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CERTIFICATE

This is to certify that the Dissertation entitled **"FORMULATION AND EVALUATION OF DICLOFENAC POTASSIUM ETHOSOMES"** submitted by **Mr. M.R.VIJAYAKUMAR** in partial fulfillment of the requirement for the degree of **Master of Pharmacy** in **Pharmaceutics** is a bonafide work carried out by him, under my guidance and supervision during the academic year 2009 – 2010 in the Department of Pharmaceutics, Madurai Medical College, Madurai-20.

I wish him success in all his endeavors.

Place: Madurai

Date: **(A.Abdul Hasan Sathali)**

FORMULATION AND EVALUATION OF DICLOFENAC POTASSIUM ETHOSOMES

Dissertation submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY, CHENNAI

in partial fulfillment of the requirement for the award of degree of

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CHAPTER- I

INTRODUCTION

The skin covers a total surface area of approximately 1.8m^2 and provides the contact between the human body and the external environment. Dermal drug delivery is the topical application of drugs to the skin in the treatment of skin diseases. This has the advantage that high concentrations of drugs can be localized at the site of action, reducing the systemic side effects. Transdermal drug delivery uses the skin as an alternative route for the delivery of systemically acting drugs [1].

The alleviation of the psychological, physical suffering and the cure or at least amelioration of disfiguring diseases such as eczema, psoriasis, icthyosis and the skin cancers are noble aims of dermal and transdermal delivery of drugs. For example, in the UK alone there are 44,000 skin carcinoma cases per year, of which 2000 are fatal (study at 2001) [2]. The main reason for many problems with transdermal drug delivery is that the impermeability of human skin limits the delivery of daily drug, for example from an acceptable sized patch approximately 10mg. How to increase this low limit for topical systems in general provides a major challenge to scientists and many university laboratories worldwide.

Transdermal route of drug administration have several advantages like circumvention of the variables that could influence gastro intestinal absorption such as P^H, food intake, gastrointestinal motility, circumvention of hepatic metabolism, constant controlled drug input and targeting of the active ingredient for a local effect. Constant drug input from the transdermal formulation decreases the variations in the drug plasma levels, reducing the side effects particularly of drugs with narrow therapeutic window.

Although the skin as a route for drug delivery can offer many advantages, the barrier nature of the skin makes it difficult for most drugs to penetrate into and permeate through it[3]. Human skin effectively inhibits drug permeation mainly because of the stratum corneum. Thus to maximize drug flux, formulations reduce the hindrance of this barrier, although sometimes drug transport via the hair follicle might also be involved. During past decades there has been wide interest in exploring new techniques to increase drug absorption through the skin. But only few drugs are currently in market in transdermal drug delivery system like clonidine, estradiol, nitroglycerine, fentanyl, testosterone, scopolamine, nicotine and oxybutinin[4-8].

The structure of stratum corneum is often compared with a brick wall, with the corneocytes as the bricks surrounded by the mortar of the intercellular lipid lamellae [9], [figure-1]. Many techniques have been aimed to disrupt and weaken the highly organized intercellular lipids in an attempt to enhance drug transport across the intact skin [10, 11]. One of the most controversial methods is the use of vehicle formulations as skin delivery systems.

CAHAPTER- II

VESICLES IN TRANSDERMAL DRUG DELIVERY- A REVIEW

The first paper to report the effectiveness of liposomes for skin delivery was published by Mezei and Gulasekharam(1980) [12,13]. Conflicting the results continued to be published concerning their effectiveness, enhancing the controversy of liposomes as dermal and transdermal drug delivery vesicles [14]. The first therapeutic using lipid vesicle on the skin was commercialized shortly before year 1990 and contained the antimycotic agent econazole. A few other, relatively simple liposome based dermal products followed [15-17]. Recently suggested, classic liposomes are of little or no value as carriers for transdermal drug delivery as they do not deeply penetrate the skin.

Intensive research led to the introduction and development of new class of lipid vesicles, the highly deformable (ultradeformable or elastic) liposomes that have been termed transferosomes [18]. Several studies have been reported that deformable liposomes were able to improve invitro and invivo skin delivery of various drugs [19-24] with efficiency comparable with subcutaneous administration [25-28].

Ethosome is a novel lipid carrier, recently developed by Touitou et al showing enhanced skin delivery.

Conventional liposomes [29,30]

Liposomes are lipid vesicles contain an aqueous volume enclosed by bilayer lipid membrane . Lipid vesicles are usually phospholipids with or without some additives, cholesterol may be included to improve bilayers characteristics of liposomes, increasing micro viscosity of the bilayers, reducing permeability of the membrane to water soluble molecules, stabilizing the membrane and increasing rigidity of the vesicles.

From the study of Mezei and Gulasekharam(1980), four to five fold triamcinolone acetonide concentrations in the epidermis and dermis with lower systemic drug levels were observed when the drug was delivered from liposomal lotion in comparison with conventional formulations. Similar findings were also observed for triamcinolone acetonide liposomal gel formulations and for progesterone and econozole[30]. Several invivo and invirto transport studies reported that conventional liposomes only enhanced skin deposition with mostly reduction in percutaneous permeation or systemic absorption of hydrocortisone [31], other corticosteroids [32], lidocaine [33], tretinoin [34] and cyclosporine [35].

Although some authors suggested conventional liposomes as suitable carriers for transdermal delivery of some drugs, it became recently evident that in most cases, classic liposomes are of little or no values as carriers for transdermal drug delivery as they do not deeply penetrate skin but rather remain confined to upper layers of the stratum corneum. Confocal microscopy studies showed that intact liposomes were not able to penetrate into granular layers of the epidermis.

An important role of the transappendageal route in improving skin delivery of drugs by liposomes was also suggested [36, 37].

Highly deformable liposomes (transferosomes)

While conventional liposomes were reported to have mainly localizing or rarely transdermal effects, deformable liposomes were reported to penetrate intact skin, carrying therapeutic concentrations of drugs but only applied under non-occluded conditions.

Transferosomes are the first generation of elastic vesicles introduced by Cevic and Blume (1992) [38]. They consist of phospholipids and an edge activator. An edge activator is often a single chain surfactant, having a high radius of curvature that destabilizes lipid bilayers of the vesicles and increases deformability of the bilayers. Sodium cholate, sodium deoxy cholate, span 60, span 65, span 80, tween 20, tween 60, tween 80 and dipotassium glycyrrhizinate were employed as edge activators. Preparation of deformable liposomes involves methods similar to those used in preparation of traditional liposomes.

Deformable liposomes as carriers

Deformable liposomes resulted in 14-17 fold increase in oestradiol flux through human cadaver epidermis with 9.2-11 fold increase in skin deposited drug. Deformable liposomes were also reported to improve both invitro skin permeation and deposition of cyclosporine A [39], methotrexate [10], melatonin [40],. It is also reported that transferosomes improve only skin deposoition of 5-fluorouracil [41] and dipotassium glycyrrhizinate [11].

Reported success of deformable liposomes to deliver macromolecules and proteins such as insulin with efficiency is comparable with subcutaneous administration. Transferosomes also reported for deliver genetic materials into mice [42], non invasive delivery of tetanus toxoid(TT) and ketoprofen transdermal delivery[43].

Niosomes as elastic vesicles

A second generation of elastic vesicles (niosomes) consisting mainly of nonionic surfactants was introduced by Van Den Bergh (1999) [33, 34]. This surfactant

based elastic vesicles were shown to be also more effective than rigid vesicles in enhancing skin penetration of various chemical entities [46-48].

Two mechanisms were proposed to improve the skin delivery of drugs by deformable vesicles. First, vesicles can act as drug carrier systems whereby intact vesicles enter the stratum corneum carrying vesicle-bound drug molecules into the skin. Second, vesicles can act as penetration enhancers, whereby vesicle bilayers enter the stratum corneum and subsequently modify the intercellular lipid lamellae. This will facilitate penetration of free drug molecules into and across the stratum corneum.

For hydrophilic drugs the penetration enhancing effect seems to play a more important role in the enhanced skin delivery than in the case of lipophilic drugs, since permeation of hydrophilic molecules tends to be relatively slower [18].

Ethosomes

Ethosome is a novel lipid carrier developed by Touitou et al showing enhanced skin delivery. The ethosomal system is composed of phospholipid, ethanol and water. Although liposomal formulations containing up to 10% ethanol and up to 15% poly propylene glycol were previously described by Foldvary et al (1993) [49], the use of high ethanol content was first described by Touitou et al (1997) for ethosomes. Due to the interdigitation effect of ethanol on lipid bilayers, it was believed that high concentrations of ethanol are detrimental to liposomal formulations. However, ethosomes which are novel permeation enhancing lipid vesicles embodying high concentration (20-45% v/v) of ethanol were developed and investigated [50].

Several studies investigated that the effect of ethanol on physiochemical characteristics of ethosomal vesicles. The reduction in vesicle size could be explained as a

result of incorporation of high ethanol concentration. Ethanol confers a surface negative net charge to the liposome which causes the size of vesicles to decrease. The size of ethosomal vesicles was reported to increase with decreasing ethanol concentration range of 20-45% v/v. The effect of phospholipid concentration on the size of ethosomal vesicles was also investigated by Touitou et al and Elsayed et al.

Ethosomes have been shown to exhibit high encapsulation efficiency for a wide range of molecules including lipophilic drugs. This could be explained by multilamellarity of ethosomal vesicles as well as by the presence of ethanol in ethosomes which allows for better solubility of many drugs. Ethosomes were reported to improve invivo and invitro skin delivery of various drugs. Contrary to deformable liposomes, ethosomes are able to improve skin delivery of drugs both under occlusive and non-occlusive conditions.

CHAPTER- III

ETHOSOMES- A REVIEW

Ethosomes is a novel lipid carrier recently developed by Touitou et al [51,52]. Ethosomal systems are novel permeation enhancing lipid carriers embodying ethanol containing lipid vesicles with interdigitated fluid bilayers. In contrast to the liposomes and deformable liposomes, ethosomes have been shown to exhibit high encapsulation efficiency, skin deposition ability and depth of skin penetration for a wide range of molecules including lipophilic drugs and are effective at delivering molecules to and through the skin. Wheather for pharmaceutical purposes, gene therapy, vaccination or cellular transformations in biomedical research the delivery of molecules through the biological membrane has become a major focus of research in recent years. Results from the intracellular delivery of the labeled phospholipids indicate that the ethosomal components themselves may penetrate the fibroblasts. The probe is found throughout the cell structure, in the membrane and in the cytoplasm.

Mechanism of enhanced drug penetration:

Ehosomes is a novel vesicular carrier showing enhanced delivery of drugs to the deeper layers of skin. The mechanism of enhanced penetration also demonstrated by the same author in 2000 [51]. The ethosomal system is composed of phopholipid, ethanol and water. Ethanol has long been known to have permeation enhancing properties. However, the permeation enhancement from ethosomes observed in this work was much greater than would be expected from ethanol alone, suggesting some kind of synergistic mechanism between ethanol, vesicles and skin lipids. In comparison to liposomes, ethosomes are less rigid, confirmed by relatively low fluorescence anisotropy of the

phosphatidylcholine probe AVPC (antryl- vinly phosphatidyl choline) as well as low T_m in differential scanning calorimetric studies. Thus the effects of ethanol which were considered harmful to classic liposomal formulations may provide the vesicles with soft flexible characteristics which allow them to more easily penetrate into deeper layers of the skin. Another contribution to the high skin penetration could be made by the interaction of ethanol and of phospholipid vesicles with stratum cornium. It has also been suggested that mixing of phospholipids with the subcutaneous lipids of the intercellular layers enhances the permeability of the skin.

The following figure also given by Touitou et al illustrates a hypothetical model of how ethosomes may enhance permeation of drugs through the stratum corneum lipids. First ethanol disturbs the organization of the stratum conium lipid bilayer and enhances its lipid fluidity. The flexible ethosomal vesicles can then penetrate the disturbed subcutaneous bilayers and even forge a pathway through the skin by virtue of their particulate nature. The release of drug and absorption by deep layers could be the result of fusion of ethosomes with skin lipids and drug release at various points along the penetration pathway. When ethosomal carriers which contain ethanol and soft small vesicles are applied to the skin, it follows stratum cornium and pilosebacious pathways.

Ethosomes

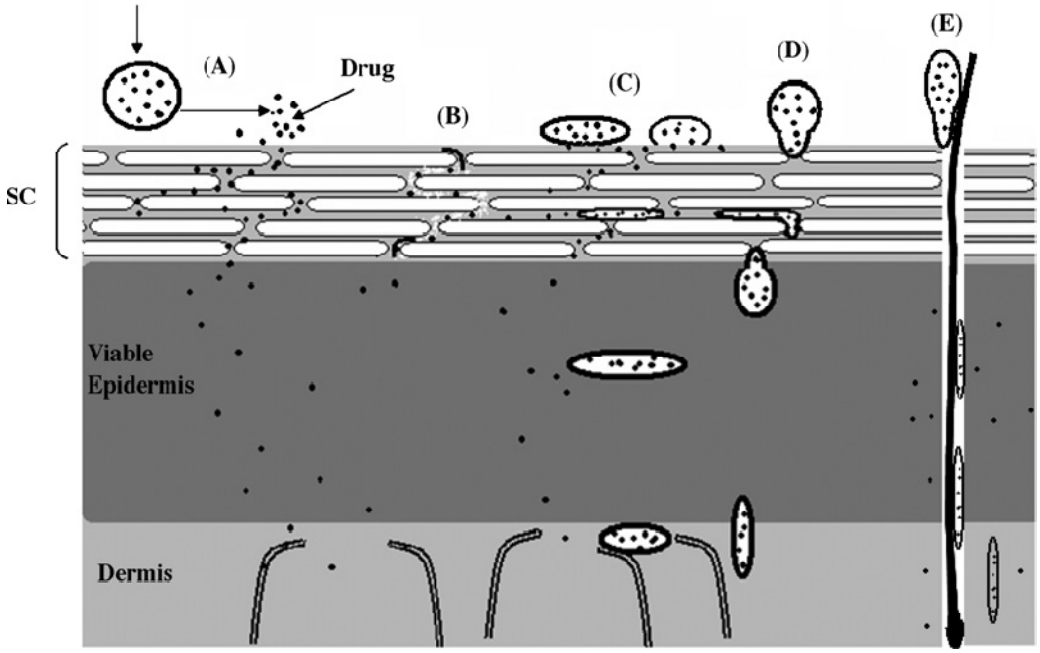


Figure-1: Possible mechanisms of action of ethosomes as skin drug delivery systems. (A) is the free drug mechanism, (B) is the penetration enhancing process of ethosome components, (C) indicates vesicle adsorption to and/or fusion with the stratum conium (SC) and (D) illustrates intact vesicle penetration into or into and through the intact skin (E) illustrates penetration of vesicles through hair follicles (modified from El Maghraby et al., 2006) [36].

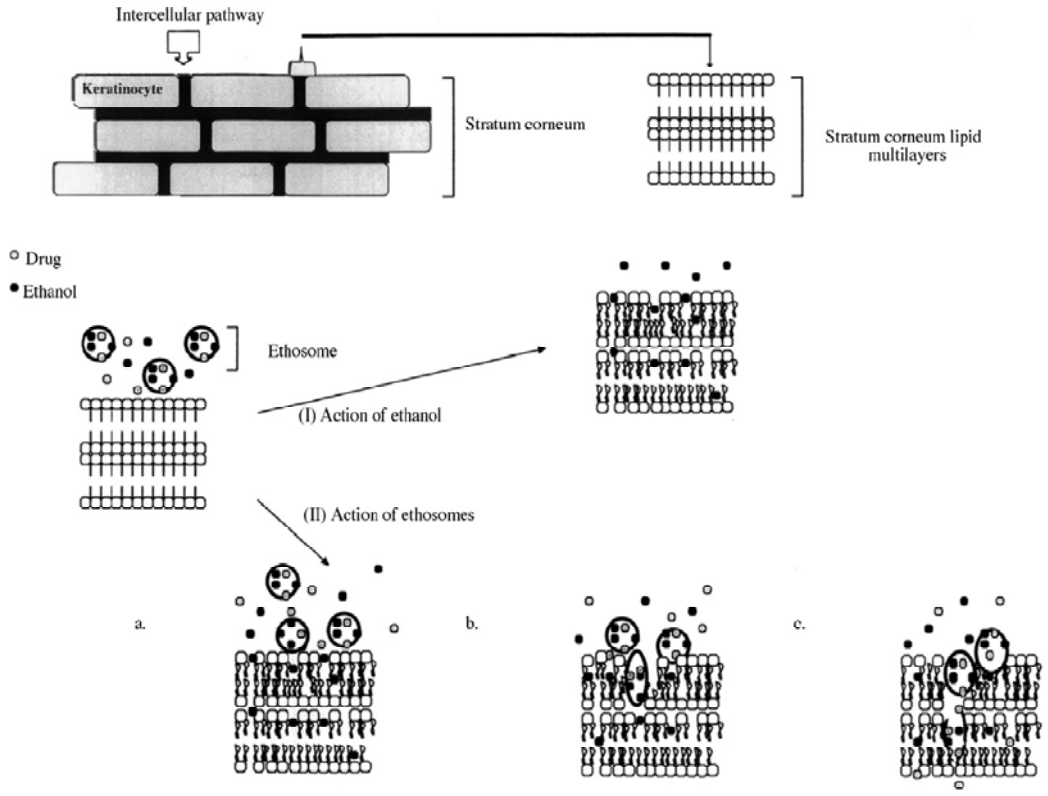


Figure-2: Proposed model for skin delivery of drugs from ethosomal system.[51]

The mechanism of ehtosomal drug delivery is reviewed by M.M.A.Elsayed et al. In his study, drug solution in 30% ethanol showed a slight, non significant cumulative drug permeated relative to the aqueous control. This could suggest that the penetration enhancing effect of ethanol is not an operating mechanism in the observed enhancement of skin delivery of drugs by ethosomes. The proposal that ethanol may increase the flexibility of the vesicles, allowing them to more easily penetrate into deeper layers of the skin, could be supported. This may be followed by structural changes in deep layers of the stratum cornium, with subsequent enhancement of drug penetration.

Preparation methods:

1. Injection to ethanolic solution method [51, 52, 53]

From the currently available references this method is first reported by E.Touitou et al. and Jain et al. In this method the drug and phosphatidyl choline is dissolved in ethanol and taken in a hermatically closed glass bottle. Double or triple distilled water heated to 30±1°C is added slowly in a fine stream to the lipid solution with constant stirring at 700rpm with a mechanical or magnetic stirrer. Mixing is continued for additional 5 minutes. The system is maintained at 30±1°C during the preparation and then left to cool to room temperature for 30 minutes. The resulting vesicle suspension is homogenized by passing through polycarbonate membrane with extruder or sonication by a probe sonicator.

2. Ethanol injection - sonication method [54]

This method is followed by zhaown et al. In this method the phospholipid is dissolved in ethanol in a glass bottle. The drug is dissolved in double distilled water and stirred by a magnetic stirrer. The glass bottle is connected to a syringe hermetically, which permits the supplement of ethanol, but avoided evaporation. After dissolution of the drug, the phospholipid – ethanol solution is added into the drug solution at a flow rate of 200µl/min, and then the mixture is finely homogenized at 50°C by sonication for 5 minutes (300 watt) by a sonifier probe type ultrasonic instrument. The obtained colloidal solution is filtered through 0.22 µm disposable filters and then the ethosomes formulation containing ethanol and water is obtained in the filtrate. All the processes are carried out under the protection of nitrogen at room temperature.

3. Classic mechanical dispersion method [55, 56]

This method is reported by Dubey et al., Fang et al. and Curic et al. In this method phosphatidyl choline is dissolved in chloroform : methanol 3:1 mixture in a clean round bottom flask followed by removal of the organic solvents using rotary flash evaporator above the lipid transition temperature to form a thin lipid film under vacuum overnight followed by hydration with different concentration of hydro ethanolic mixture containing drug with 60rpm for 1 hour at the corresponding temperature. The preparation vortexed followed by sonication at 4^{o}C using a probe sonicator.

The same method is followed by Tadros et al. with emulsification by sonication followed by solvent evaporation in rotary flash evaporator. In this procedure the lipid dissolved in small volume of Di ethyl ether : Choloroform 1:1 mixture in a round bottom flask. An aqueous phase containing water soluble drug is added to the organic phase such that organic to aqueous phase ratio was 5:1. The mixture is then sonicated for 10 minutes. A stable white emulsion produced from which the water and organic solvent mixture slowly evaporated at 55^{o}C using rotary flash evaporator until a thin film formed on the wall of the flask. The resulting film kept vacuum to eliminate the traces of organic solvent. This film was hydrated with different concentration of hydro ethanolic mixture for 1 hour with rotation. The resulted formulation left at room temperature for 1 hour and then sonicated for 20 minutes at 4^{o}C.

Vesicular characterisation:

1. Vesicular size and zeta potential [51, 52, 53]

This can be determined by Dynamic Light Scattering (DLS) technique using a computerized inspection system. The water ethanol solution of the same proportion of the

formulation or saline or P^H: 6.8 phosphate buffer saline (PBS) is used as sample diluents. The vesicular suspension is mixed with the appropriate medium and the measurements are taken.

2. Visualization for vesicular shape and surface morphology [51, 52, 53]

Transmission Electron Microscope (TEM) is used for visualization of ethosomal vesicles. The vesicular suspension is placed on carbon coated grid or copper grid and the material excess is removed with a filter paper. This is stained with aqueous solution of phosphotungstic acid or 2% uranyl acetate solution. Finally the grid is semi dried and examined under Transmission electron microscope.

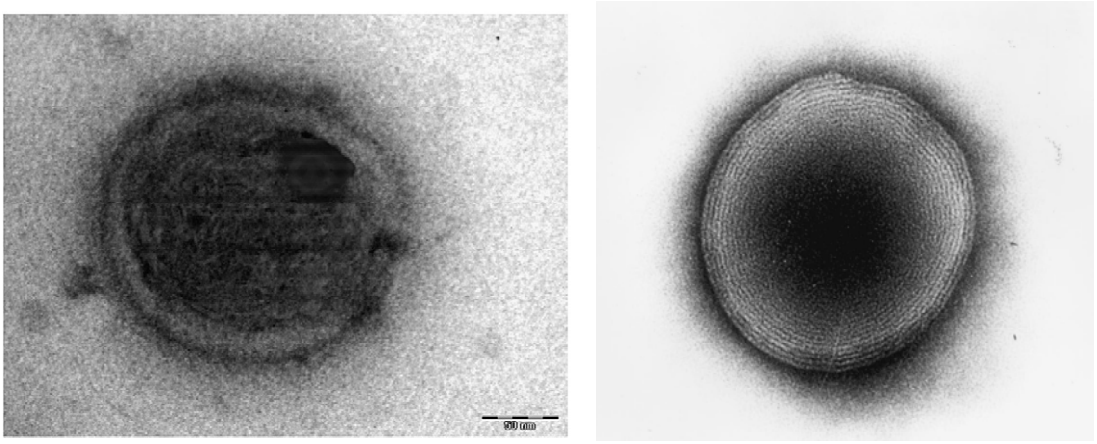


Figure- 3 Visualization of ethosomal vesicles TEM (a) magnification 315 000 [51] (b) magnification 1,10,000 [55].

For vesicle characterization, freeze fracture transmission electron microscopy also used. After centrifugation the samples at 30,000g for 30 min at room temperature, are examined by means of the freeze fracture microscopy technique: samples are impregnated with 30% glycerol and then frozen in partially solidified Freon 22, freeze fractured in a freeze fracture device (-105^{o}C, 10–6 mm Hg) and replicated by evaporation

from a platinum/carbon gun. The replicas are extensively washed with distilled water, picked up onto Formvar-coated grids and examined with a transmission electron microscope (TEM).

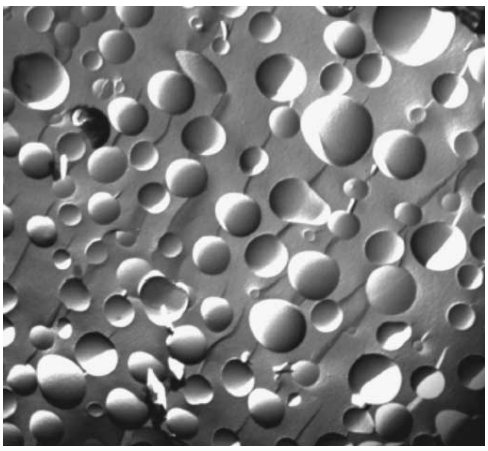


Figure-4: Freeze fracture electron micrographs of ethosome formulation containing ammonium glycyrrhizinate [64]

Scanning Electron Microscope (SEM) is also used to characterize the surface morphology of the ethosomes. One drop of ethosomal suspension is mounted on a clear glass stub, it is air dried and coated with gold and visualized under Scanning electron microscope.

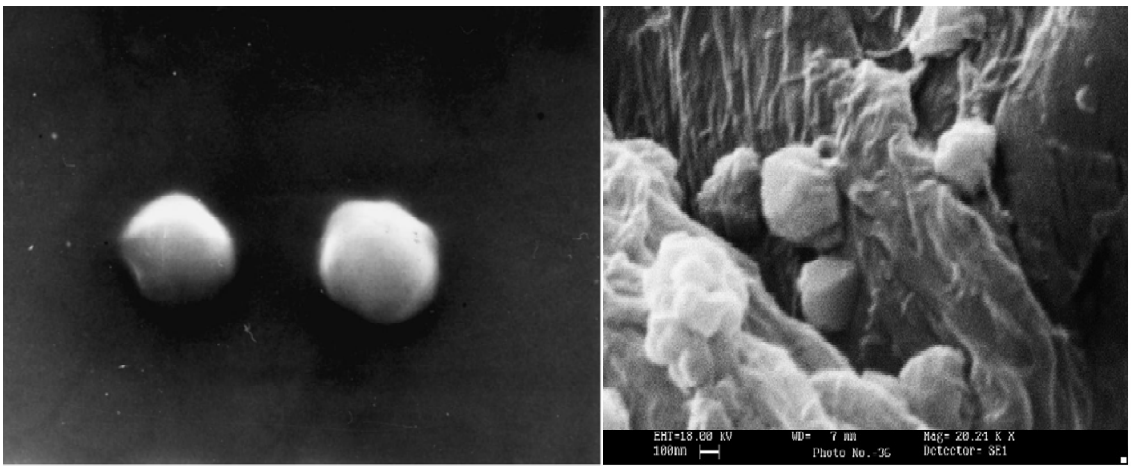


Figure-5: Visualization of ethosomal vesicles by SEM. (a) testosterone ethosomes[51] (b) Methotrexate ethosomes [55]

3. Drug entrapment efficiency:

Drug entrapment efficiency can be determined by 3 methods,

(a) Ultra centrifugation method

(b) Sephadex G-50 minicolumn centrifugation method

(c) Dialysis method

(a) Ultra centrifugation method [51, 52]

After preparation, the ethosomal formulation is centrifuged at $4^{o}C$ at a speed ranging from 15,000 rpm to 56,000 rpm for a time ranging from 30 minutes to 4 hours by ultra centrifuge. The required rotation speed of the centrifuge depends upon the individual formulation characteristics. Usually higher revolution speed will cause the separation of the supernatant in less time duration. The amount of drug in supernatant and ethosomal pellet is determined. The amount of drug in the ethosomal sediment is determined after lysing the vesicles by triton X-100 0.5% w/w. From this entrapment efficiency is determined by

$$E.E = D_E / D_E - D_S \quad \text{or} \quad E.E = D_T - D_S / D_T$$

Where,

D_E ─ Amount of drug in the etosomal sediment

D_S ─ Amount of drug in the supernatant

D_T ─ Theoretical amount of drug used to prepare the formulation.

(b) Sephadex G-50 minicolumn centrifugation method [55, 57]

The prepared ethosomal vesicles separated from the free (unentrapped) drug by a sephadex G-50 minicolumn centrifuge. The collected vesicles were lysed by Triton X-100 0.5% W/V and entrapped efficiency is determined by the formula explained in ultra centrifugation method.

(c) Dialysis method [58]

Cellulose acetate dialysis membrane is kept in saline for 1 hour before dialysis to ensure complete wetting of the membrane. The formulation is placed in the dialysis bag, which is then transferred into the receiver medium stirred by magnetic stirrer. Samples are withdrawn at regular time interval with the replacement of receptor volume. After 4 hours when free drug is separated, the vesicles are lysed using Triton X-100 and analyzed for drug content.

4. Depth of skin penetration [51, 52]

Depth and mechanism of skin penetration of fluorescent probe loaded ethosomes is investigated using Confocal Lasser Scanning Microscopy(CLSM). The probe loaded vesicles are first passed through the sephadex G-50 minicolumn to separate the unentrapped probe and formulation is applied non-occlusively for 8 hours to the dorsal skin of nude albino rat. The rat is then sacrificed by heart puncture; dorsal skin was gently teared off any adhering fat and/or subcutaneous tissue. The skin is sectioned into pieces of $1mm^2$size.The full skin thickness is optically scanned at different increments (usually10-20μm) through the z axis of a CLS Microscope. Optical excitation is carried out with a 488 nm for Rhodamine red (RR) at 527 nm for D-289 (4-(4-di ethyl amino) stearyl-N- methyl pyridinium iodide) and at 488 nm for calcein. This entire study can also

performed in invitro by fixing the rat skin in franz diffusion cell and applied the probe loaded ethosomes. After the contact period the skin is washed and analyzed by laser scanning.

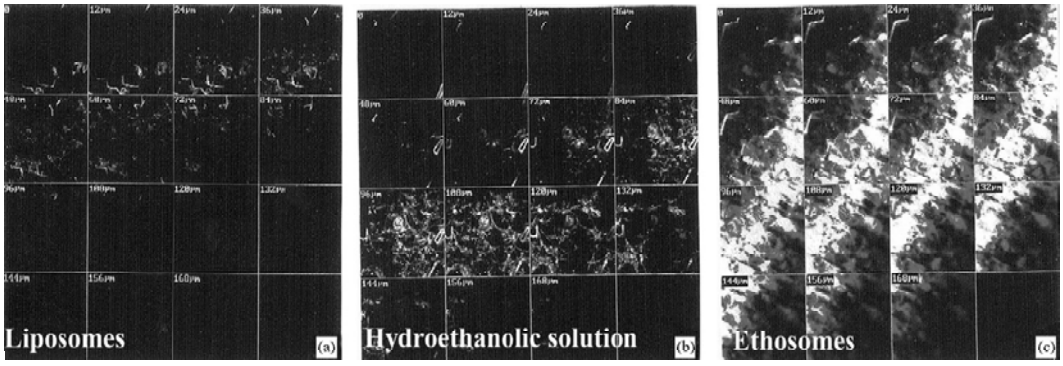


Figure-6: Confocal laser scanning microscopy micrographs of mouse skin, after application of the fluorescent probe D-289 in traditional liposomes, hydroethanolic solution and ethosomes [51]

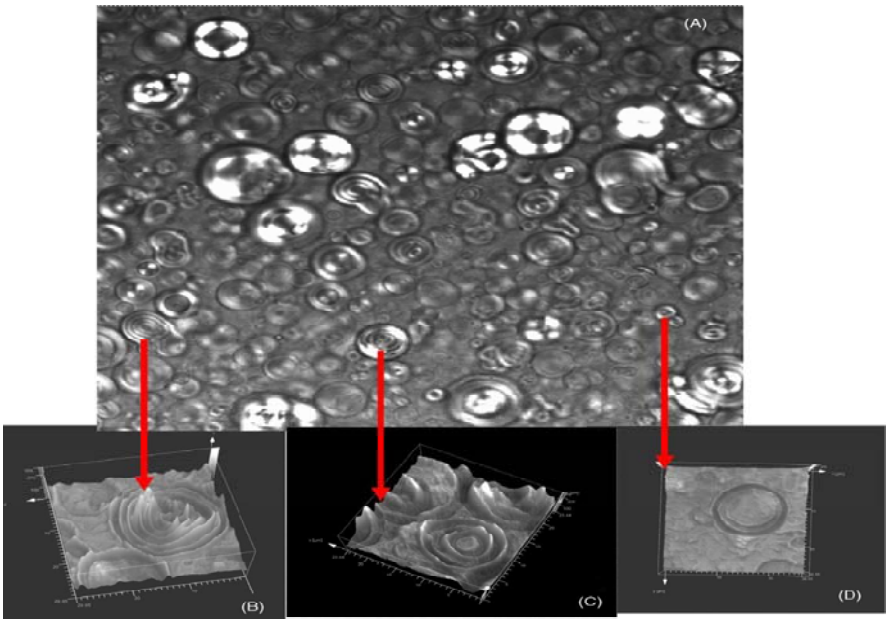


Figure-6: Microphotographs corresponding to DPPC multilamellar liposomes by CLSM using transmitted channel (A) the whole field; (B) six lamellae; (C) three lamellae. (D) unilamellar vesicle

(5) Invitro drug release studies (skin permeation studies) [51, 52, 53]

Franz diffusion cell is mostly used for invitro skin permeation studies. Valia-Chien cell was used for permeation study of Minoxidil and Trihexy phenidyl Hcl ethosomes by Touitou et al. Skin of new born mice, abdominal and dorsal skin of male nude mouse, rat skin, male albino rabbit pinna skin taken from inner ear, dermatomed human cadaver skin from abdominal areas and human abdominal skin obtained by plastic surgery were used for the permeation studies. Synthetic semi permeable membranes made of cellulose material also used for the invitro drug release studies.

To get the skin from animals, the animal is sacrificed by chloroform inhalation. The hair of test animals were carefully trimmed short (<2mm) with scissors and skin is separated from the underlying connective tissue with a scalpel. The excised skin was placed on the skin is gently teased off for any adhering fat and/or subcutaneous tissue. The obtained skin is mounted between the donor and receptor compartment with the stratum conium side facing upward into the donor compartment. The prepared Franz diffusion cell is either used immediately after preparation or after stored in the refrigerator (4^oC) in order to hydrate the skin overnight. Caution is taken to remove all bubbles between the underside of the skin and the acceptor solution.

Isotonic phosphate buffer solution like phosphate buffered saline P^H: 6.5, P^H: 7.4 and citrate phosphate buffer P^H: 7.4 and hydroethanolic solution were used as receptor solution. In some studies 0.11% w/v of formaldehyde containing medium also used. During this studies 32 ± 1^oC (skin surface temperature) is maintained. Some authors maintained human body temperature (37 ± 1^oC) as itself. Stirring is maintained by a magnetic stirrer throughout the experiment. The ethosomal formulation is applied on the skin in donor compartment, which is then covered with a parafilm to avoid any evaporation process. Samples are withdrawn through the sampling port of the diffusion

cell at predetermined time intervals over 12 hours to 24 hours and analyzed for drug content by respective analytical techniques. The receptor phase is immediately replenished with equal volume of fresh medium maintained at the same temperature as that of receptor compartment. Skin conditions are maintained throughout the experiments.

(6) Skin retention study:

The amount of drug retained in the skin is determined at the end of the invitro permeation experiment. The skin is washed using a cotton cloth immersed in methanol. The drug retention is determined by two methods.

(a) Skin destruction method: The sample of skin is weighed, cut with scissors, positioned in a glass homogenizer containing 1ml methanol and homogenized for 5minutes with an electric stirrer. The resulting solution is centrifuged for 10 minutes at 7000rpm. The supernatant is analyzed for drug content by suitable analytical technique [59, 60, 61].

(b) Skin extraction method: After completion of invitro permeation study the receptor content is completely removed and replaced by 50%v/v ethanol in distilled water and kept for further 12 hours, then the drug content is determined. This receptor solution diffused through the skin, disrupting any liposome structure and extracting deposited drug from the skin, thus giving a measure of skin deposition [51, 52, 55, 62].

(7) Skin irritancy studies [57]

Irritancy of different formulation is determined in male albino rabbits. The animals divided into groups as required. One group receives ethosomal formulation and one group receives hydroethanolic solution of drug having an ethanol concentration used in ethosomal formulation. The score of erythema is determined. This study was reported by V.Dubey et al for melatonin ethosomes and demonstrated that ehanol present in the

ethosomal formulation is not able to act as a skin erythema inducing agent, even though present in high concentration.

(8) Differential scanning calorimetry (DSC) measurements [[51, 63]

The transition temperature (T_m) of vesicular lipids is measured using modulated differential scanning colorimetry with programmed heating rate of 10^oC/min under constant nitrogen stream within a range of -50^o to $+50^oC$. The amount of sample usually carried out this experiment is 20±5mg. Calorimetric studies demonstrate low T_m values for ethosomal system as compared to liposomes suggesting a fluidizing effect of ethanol on phospholipid bilayers. Thus ethosomes considered as a soft liquid state vesicles with fluid bilayers. Further T_m of the drug loaded ethosomal system is similar to that of ethosomal systems, suggesting presence of drug in ethosomal core. If this not similar indicates presence of drug in bilayer.

(9) Storage and physical stability of ethosomes [51]

The vesicular suspensions are kept in sealed vials after flushing with nitrogen and stored at different temperatures $4±2^oC$ (actual storage temperature), $25±2^oC$ (room temperature). The stability of ethosomes was assessed quantitatively by monitoring size and morphology of the vesicles overtime using dynamic light scattering technique and TEM. For assessing the skin permeability of stored ethosomal system, confocal laser microscopic studies were performed.

CHAPTER- 4

LITERATURE REVIEW

1. E.Touitou et al., developed minoxidil and testosterone ethosomes. He proved ethosomal systems were much more efficient at delivering a fluorescent probe to the skin in terms of quality and depth than either liposomes of hydroethanolic solution. The ethosomal system dramatically enhanced the skin permeation of minoxidil invitro compared with either ethanolic of hydroethanolic solution or phospholipid ethanolic micellar solution of minoxidil. In addition, the transdermal delivery of testosterone from ethosomal patch was greater both invitro and invivo than from commercially available patches. Skin permeation was demonstrated in diffusion cell experiments. Ethosomal system composed of soy phosphatidyl choline 2%w/v, ethanol 30%v/v and water were shown by electron microscopy to contain multilamellar vesicles. ^{31}P-NMR studies confirmed the bilayer configuration of the lipids. Calorimetry and fluorescence measurements suggested that the vesicular bilyers are flexible, having a relatively low T_m and fluorescence anisotropy compared with liposomes obtained in the absence of ethanol. Dynamic light scattering was used in the average vesicle size measurement [50].

2. N.Dayan and E.Touitou characterized an ethosomal carrier containing Trihexyphenidyl Hcl and investigated the delivery of drug from ethosomes versus classic liposomes. As the drug concentration increased from 0 to 3% the size of the vesicles decreased from 154nm to 94nm due to surface activity of trihexiphenidyl, measured in his work. Zeta potential also increased from -4.5 to +10.4 as increasing the concentration from 0 to 3% of drug. In contrast, trihexyphenidyl liposomes were much larger and their charge was not affected by the drug. Ethosomes had a greater ability to deliver entrapped fluorescent probe to the deeper layers of skin. The flux of drug through nude mouse skin

from ethosomes was 87, 51 and 4.5 times higher than from liposomes, Phosphate buffer and hydroethanolic solution respectively. The drug retention in the skin at the end of the 18-hour release experiment was statistically significantly greater than from liposomes or a control hydroethanolic solution. The efficient drug delivery with the long term stability of ethosomes made this system a promising candidate for transdermal delivery of trihexyphenidyl [51].

3. E.Touitou et al., investigated the efficiency of transcellular delivery in to swiss albino mice 3T3 fobroblasts of molecules with various physico-chemical characteristics from ethosomes. The probes chosen were 4-(4-diethlyamino) styryl- N- methyl pyridinum iodide (D-289), rhodamine red (RR) and fluorescent phosphatidyl choline (PC^{*}). The penetration of these fluorescent probes into fibroblasts and nude mice skin was examined by CLSM and flocytometry analysis. CLSM micrographs showed that ethosomes facilitated the penetration of all probes into the cells. But from hydroethanolic solution or classic liposomes, almost no fluorescence was detected. Enhanced delivery of molecules from the ethosomal carrier was also observed in permeation experiments with the hydrophilic calcein and lipophilic RR to whole nude mouse skin. Calcein penetrated the skin to a depth of 160, 80 and 60µm from ethosomes, hydroethanolic solution and liposomes were 150, 40, and 20 AU respectively. The highly efficient delivery exhibited together with its non-toxicity, made this system a promising and chemical compounds to both skin and cultured cells [52].

4. Elisabetta Esposito et al., produced ethosomes by the method described by Touitou et al, addition of an aqueous phase to an ethanol solution of soy phosphatidylcholine 5%w/w and azelaic acid under mechanical stirring. Liposomes were obtained by the same composition without ethanol with the reverse phase evaporation method. In order to obtain homogenously sized vesicles, both ethosomal and liposomal dispersions were

extruded through polycarbonate membrane with400nm and 200nm pore size, vesicle size was measured by photon correlation spectroscopy and vesicles morphology was characterized by freeze-fracture scanning electron microscopy. Free energy measurements of the vesicle bilayers were conducted by differential scanning calorimetry. Diffusion studies of ethosomal and liposomal incorporated gel was investigated by a Franz cell assembled with synthetic membranes. The release rate was more rapid from ethosomal systems than from liposomal systems. In particular, ethosomes produced by the highest ethanol concentration released azelaic acid more rapidly [64].

5. D.Ainbinder and E.Touitou designed and tested a testosterone non patch formulation using ethosomes for enhanced transdermal absorption. The ethosomal formulation was characterized by transmission electron microscopy and dynamic light scattering for structure and size distribution and by ultracentrifugation for entrapment capacity. The systemic absorption of drug from this formulation in rats was compared with a currently used gel (Androgel®). Further, theoretical estimation of testosterone blood concentration following ethosomal application in men was made. For this purpose, invitro permeation experiments through human skin were performed to establish testosterone skin permeation rules. This work showed that the ethosomal could enhance testosterone systemic absorption and also be used for designing new products to solve the weakness of the current testosterone replacement therapies [55].

6. Biana Godin and E.Touitou designed and characterized Erythromycin ethosomes and their antibacterial efficiency was evaluated invitro and invivo. TEM, CLSM, DLS, DSC studies performed and ultra centrifugation tests indicated erythromycin etrhosomes are small unilamellar soft vesicles encapsulating 78.6% of erythromycin. Susceptibility studies conducted on 3 bacterial strains. Ethosomal erythromycin applied to the skin of

ICR mice inoculated with 10^7cfu S.aureus ATCC29213 resulted in complete inhibition of infection. On the contrary, when hydroethanolic solution of erythromycin was applied, deep dermal and subcutaneous abscesses developed within 5 days after challenge. For these animals histological examination showed necrosis, destroyed skin structures and dense infiltrates of neutrophils and macrophages. These findings showed that ethosomes are efficient carriers for erythromycin delivery to bacteria localized within the deep skin strata for eradication of staphylococcal infections [63].

7. J.Y.Fang et al., developed catechins encapsulated liposomes incorporating anionic surfactants and ethanol. Liposomes were characterized for size, zeta potential and entrapment efficiency. Both invitro and invivo skin permeation performed using nude mouse skin as a model. Incorporation of anionic surfactants such as deoxy cholic acid and dicetyl phosphate in the liposomes in the presence of 15% ethanol increased the (+)-catechin permeation by five to seven fold as compared to the control. The stability and invitro transepidermal water loss test indicated the safety of the practical use of liposomes developed in this study [59].

8. M.M.A.Elsayed et al., investigated the possible mechanisms by which deformable liposomes and ethosomes improve skin delivery of ketoprofen under non-occlusive conditions. Invitro permeation and skin deposition behavior of deformable liposomes and ethosomes , having ketotifen only inside the vesicles, only outside the vesicles and both inside and outside the vesicles was studied using rabbit pinna skin. Results suggested that both the penetration enhancing effect and the intact vesicle permeation into stratum corneum might play a role in improving skin delivery of drugs by deformable liposomes and that penetration enhancing effect was of greater importance in case of ketoprofen. Regarding ethosomes, results indicated that ketoprofen should be incorporated in

ethosomal vesicles for optimum skin delivery. Ethosomes were not able to improve skin delivery of non entrapped ketoprofen [62].

9. V.Dubey et al., 2007 (Eur. J. Pharm. Sci) evaluated the transdermal potential of novel ethanolic liposomes (ethosomes) bearing melatonin having poor skin permeation and long lag time. TEM, SEM and dynamic light scattering defined ethosomes as spherical, unilamellar structures; nanomeric size range. Entrapment efficiency was found to be 70.71±1.4% stability profile assessed for 120 days revealed very low aggregation and grouth in vesicular size. Ethosomal carriers showed an enhanced transdermal flux of 59.2±1.22μg/cm^2/h and decreased lag time of 0.9 hours across human cadaver skin. FT-IR data revealed a greater mobility of skin lipids on application of ethosomes as compared to that of ethanol or plain liposomes. Confocal lasser scanning microscopy revealed an enhanced permeation of rhodamine red loaded formulations to the deeper layers of the skin (240nm). Further, a better skin tolerability of ethosomal suspension on rabbit skin suggested that ethosomes may offer a suitable approach for transdermal delivery of melatonin [60].

10. V.Dubey et al., 2007(J. Control. Release) investigated the transdermal potential of novel vesicular carrier, ethosomes bearing methotrexate, an anti psoriatic, anti neoplastic, highly hydrosoluble agent having limited transdermal permeation. Methotrexate loaded ethosomes were prepared, optimized and characterized for vesicular shape and surface morphology, stability, invitro human skin permeation and vesicle-skin interaction. The formulation having 3% phospholipid content and 45% ethanol showing the greatest entrapment about 68.71±1.4 and optimal nanomeric size range about 143±16nm was selected for further transepidermal permeation studies. Stability profile for 120 days, revealed very low aggregation and growth in vesicular size. Ethosomal carriers provided an enhanced transdermal flux of 52.2±4.3422μg/cm^2/h and decreased lag time of 0.9 hour

across human cadaver skin. Confocal lasser scanning microscopy revealed an enhanced permeation of rhodamine red [61].

11. Subheet Jain et al., investigated the mechanism for improved intercellular and intracellular drug delivery from ethosomes using visualization techniques and cell line study. Ethosomal formulations were prepared using lamivudine as model drug and characterized invitro, exvivo and invivo. TEM, SEM and fluorescence microscopy were employed to determine the effect of ethosome on ultra structure of skin. The optimized ethosomal formulation showed 25 times higher transdermal flux (68.4±3.522µg/cm^2/h) across the rat skin as compared with that of lamivudine solution (2.8±0.222µg/cm^2/h). Results of cellular uptake of ethosomes (85.7±4.522µg/cm^2/h) as compared with drug solution (24.9±1.922µg/cm^2/h) [57].

12. M.I.Tadros et al., compared the transdermal delivery of solbutamol sulphate, a hydrophilic drug used as a bronchodilator, from ethosomes and classic liposomes containing different cholesterol and dicetyl phosphate concentrations. All the ethosomal systems were characterized for shape, particle size and entrapment efficiency percentage by image analysis optical microscopy, laser diffraction and ultra centrifugation respectively. Invitro drug permeation via a synthetic semipermeable membrane or skin from newborn mice was studied in Franz diffusion cells. The selected formulations were incorporated into pluronic F-127 gels and evaluated for both drug permeation and mice skin deposition. The vesicle size was significantly decreased by decreasing cholesterol concentration and increasing dicetyl phosphate and ethanol concentrations. The entrapment efficiency percentage was significantly increased by increasing cholesterol, dicetyl phosphate and ethanol concentrations. Invitro permeation studies of the prepared gels containing the selected vesicles showed that ethosomal systems were much more

efficient at delivering solbutamol sulphate into mice skin than were liposomes or aqueous or hydroethanolic solutions [55].

13. Zhaowu et al., evaluated the preparation of matrine ethosomes and the percutaneous permeation invitro and the ant-inflammatory activity invivo in the rat skin. The matrine ethosomes were prepared by the ethanol injection-sonication method. The particle size, entrapment efficiency was determined by laser particle size analyzer and ultracentrifugation respectively. Anti-inflammatory activity invivo was determined by a reflection spectrophotometer. Average particle size was in the range of 50-200nm with a narrow size distribution and entrapment efficiency was in the range of 40-90%. Within 24 hours the cumulative permeation quantity is 60.5% and with no permeation quantity is 60.5% and with no permeation lag time. Matrine ethosomes able to disappear more rapidly the induced erythema than non ethosomal formulations. Matrine ethosomes can increases the percutaneous permeation of matrine in the experiment invitro and improve the anti-inflammatory activity of matrine invivo in rat skin [56].

14. Massimo Fresta et al., evaluated the various ethosomal suspensions made up of water, phospholipids and ethanol at various concentrations for their potential application in dermal administration of ammonium glycyrrhizinate, a useful drug for the treatment of various inflammatory-based skin diseases. Physicochemical characterization of ethosomes was carried out by photon correlation spectroscopy and freeze fracture electron microscopy. The percutaneous permeation of ammonium glycyrrhizinate ethosomes was evaluated in vitro through human stratum corneum and epidermis membranes by using Franz's cells and compared with the permeation profiles of drug solutions either in water or in a water–ethanol mixture. Ethosomal suspensions had mean sizes ranging from 350 nm to 100 nm as a function of ethanol and lecithin quantities, i.e.,high amounts of ethanol and a low lecithin concentration provided ethosome

suspensions with a mean size of approximately 100 nm and a narrow size distribution. In vitro and in vivo experiments were carried out by using an ethosome formulation made up of ethanol 45% (v/v) and lecithin 2% (w/v). The ethosome suspension showed good skin tolerability in human volunteers, also when applied for a long period (48 h). Ethosomes elicited an increase of the in vitro percutaneous permeation of both methylnicotinate and ammonium glycyrrhizinate. Ethosomes were able to significantly enhance the anti-inflammatory activity of ammonium glycyrrhizinate compared to the ethanolic or aqueous solutions of this drug [54].

CHAPTER-V

SCOPE OF WORK

Nonsteroidal anti-inflammatory agents (NSAIDs) are class of drugs which budded from the bark of willow in the mid-eighteenth century. Now a days, there has been a rapid increase in the number of products that have been designed to deliver NSAIDs. These include creams, gels, and more complex transdermal systems. A number of approaches have been continuousely investigated so as to enhance dermal delivery by use of prodrugs, ultrasound, ionotophorosis and microneedles. But the choice of the most appropriate drug depends on a number of factors which includes its potency, its ability to permeate the stratum corneum, its lack of local skin toxicity and stability towards metabolizing enzymes present on the skin surface.

Diclofenac potassium is one of the NSAID widely used for musculoskeletal complaints, especially arthritis, rheumatoid arthritis, polymyositis, dermatomyositis, osteoarthritis, dental pain, spondylarthritis, ankylosing spondylitis, gout attacks, and pain management in cases of kidney stones and gallstones. An additional indication is the treatment of acute migraines. Diclofenac potassium is used commonly to treat mild to moderate post-operative or post-traumatic pain, particularly when inflammation is also present, and is effective against menstrual pain and endometriosis. It can also be used to reduce menstrual pain and dysmenorrhea.

Oral dose of Diclofenac potassium causes an increased risk of serious gastrointestinal adverse events including bleeding, ulceration and perforation of the stomach or intestines which can be fatal. These events can occur at any time during use

and without warning symptoms. This drug may also cause an increased risk of serious cardiovascular thrombotic events, myocardial infarction and stroke.

Due to the presence of these oral adverse effects, necessitates the need for investigating other routes of drug delivery fo Diclofenac Potassium. Transdermal delivery of the drug can improve its bio activity and transdermal effect, reduce the side effects and enhance therapeutic efficacy. This can be achieved only when the drug has entered the lower layers of the skin, then only it can be absorbed by blood and transported to the site of action, or penetrate deeper in to areas where inflammation occurs. Pure drug or liposomal formulations not reach the lower layers of the skin.

Ethosome, a novel liposome, is especially suitable for topical and transdermal administration carrier. Compared to other liposomes, the physical and chemical properties of ethosomes make the delivery of the drug through the stratum corneum in to a deeper layer efficiently or even into the blood circulation. Diclofenac Potassium is a water soluble drug and generally the entrapment efficiency of the ethosomes of a water soluble drug is higher than that of the other vesicle formulation. So the Diclofenac potassium ethosomal formulation may be better than other transdermal or topical formulation of Diclofenac potassium.

CHAPTER- VI

PLAN OF WORK

PART –I

1. Determination of λ_{max} of Diclofenac Potassium.
2. Calibration curve for the drug in phosphate buffer saline P^H: 7.4.

PART-II

1. Formulation of Diclofenac Potassium ethosomes using different concentration of phosphatidylcholine and ethanol by classic mechanical dispersion method.

PART-III

1. Determination of drug entrapment efficiency by centrifugation method.
2. Invitro release characteristics of ethosomes in phosphate buffer saline P^H: 7.4 using synthetic semipermeable membrane.

PART-IV

1. Formulation of Diclofenac Potassium liposomes and determination of drug entrapment efficiency.

PART-V

1. Invitro release characteristics of the selected ethosomal formulation, liposomal formulation, hydroethanolic drug solution and phosphate buffer saline P^H: 7.4 drug solution through rat membrane.
2. Estimation of drug retention in the rat skin by ethanolic extraction method after 12 hours of invitro skin permeation study.

PART-VI

1. Morphological studies of ethosomes using scanning electron microscopy.

PART-VII

1. Determination of vesicular size distribution of ethosomal and liposomal formulations using dynamic light scattering (DLS) technique.

PART-VIII

1. IR studies to determine the interaction between ethosomal membranes with drug.

PART-IX

1. Stability studies of ethosomal formulations at refrigerated temperature and room temperature.

PART-X

1. Formulation of gel containing Diclofenac Potassium ethosomes.

PART-XI

1. Pharmacodynamic studies to compare the ethosomal gel formulation and marketed gel formulation.

CHAPTER-VII

MATERIALS AND EQUIPMENTS

MATERIALS USED

1. Drug- Diclofenac potassium - Apex Laboratories
2. Phospholipon 90 - Phospholipid GmbH
3. Ethanol - Ranchem
4. Chloroform - Ranchem
5. Methanol - Ranchem
6. Potassium dihydrogen phosphate - Nice chemicals
7. Di sodium hydrogen ortho phosphate - Qualigens
8. Sodium chloride - Central drug house
9. Dialysis membrane 50 LA 387 - Hi media

EQUIPMENTS USED

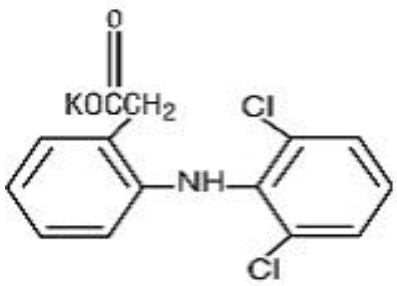
1. Rotary Flash Evaporator - Super fit rotary flash evaporator
2. Ultra Sonicator - Vibronic's Ultrasonic processor
3. High speed cooling centrifuge - Remi R-24
4. Electronic Balance - A&D Company, Japan
5. Magnetic Stirrer - MC Dalal & co
6. Mechanical stirrer -Scientific industries
7. UV Visible Spectrophotometer - UV Pharma Spec 1700, Shimadzu
8. Refrigerator - Kelvinator

CHAPTER- VIII

DRUG PROFILE

Diclofenac Potassium, a phenyl acetic acid derivative, is a non-steroidal anti-inflammatory drug (NSAID). It is widely used for anti-inflammatory and analgesic effects in the systemic treatment of acute and chronic rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. The drug is freely soluble in water and has the half life of 1.2 to 2 hours. Diclofenac Potassium is almost absorbed from the GI tract and undergoes extensive first pass metabolism in liver about 50 to 60% [65].

STRUCTURAL FORMULA [66]



EMPIRICAL FORMULA

$C_{14}H_{10}Cl_2KNO_2$

CHEMICAL NAME

2-[(2, 6-dichlorophenyl) amino]benzeneacetic acid, monopotassium salt.

MOLECULAR WEIGHT

334.25

DESCRIPTION

Faintly yellowish white to light beige, virtually odorless, slightly hygroscopic crystalline powder

CHEMICAL PROPERTIES

Solubility:

It is freely soluble in methanol, soluble in ethanol and water, and practically insoluble in chloroform and in dilute acid.

Partition co-efficient:

The n-octanol/water partition coefficient is 13.4 at pH 7.4 and 1545 at pH 5.2. It has a single dissociation constant (P^{ka}) of 4.0 ± 0.2 at 25°C in water.

MECHANISM OF ACTION[66]

The primary mechanism responsible for its anti-inflammatory, antipyretic, and analgesic action is inhibition of prostaglandin synthesis by inhibition of cyclooxygenase (COX) and it appears to inhibit DNA synthesis.

PHARMACOKINETICS [66, 67]

- Bioavailability: 100%
- Protein binding: More than 9%
- Metabolism: extensive hepatic metabolism
- Half life: 1.2 to 2 hour (35% of the drug enters entrohepatic recirculation)
- Excretion: Biliary excretion, only 1% in urine

Absorption:

Diclofenac Potassium is almost completely absorbed form the GI tract: However, the drug undergoes extensive first pass metabolism in the liver, with only about 50 to 60% of a dose of Diclofenac Potassium reaching the systemic circulation as unchanged drug. It is also absorbed into systemic circulation following rectal administration and percutaneously following topical application to the skin as gel.

Distribution:

Diclofenac Potassium is distributed into synovial fluid, achieving peak synovial fluid concentrations about 60 to 70% of those attained in plasma following oral administration.

Metabolism:

The exact metabolism of Diclofenac Potassium has not been fully elucidated, but the drug is rapidly and extensively metabolized in the liver. Diclofenac Potassium undergoes extensive hydroxylation and subsequent conjugation with glucuronic acid, taurine amide, sulfuric acid and other biogenic ligands.

Elimination:

Following oral or I.V administration of the drug in healthy adults, about 50 to 70% of a dose is excreted in urine and about 30 to 35 is excreted in feces within 96 hours.

INDICATIONS [68]

- For treatment of primary dysmenorrhea
- For relief of mild to moderate pain

- For relief of the signs and symptoms of osteoarthritis
- For relief of the signs and symptoms of rheumatoid arthritis

DOSE

For rheumatoid arthritis and osteoarthritis, 100 to 200mg of dose given as delayed release tablets. For ankylosing spondylitis 75 to 125mg of daily dose is required. In patients with dental extraction or gynecologic surgery pain 50 to 100mg doses of Diclofenac Potassium is prescribed, followed by 50mg every 8 hours.

ADVERS EFFECTS

- Gastrointestinal experiences including: abdominal pain, constipation, diarrhea, dyspepsia, flatulence, gross bleeding/perforation, heartburn, nausea, GI ulcers (gastric/duodenal), and vomiting.
- Abnormal renal function, anemia, dizziness, edema, elevated liver enzymes, headaches, increased bleeding time, pruritis, rashes, and tinnitus.
- Body as a Whole: fever, infection, sepsis
- Cardiovascular System: congestive heart failure, hypertension, tachycardia, syncope
- Digestive System: dry mouth, esophagitis, gastric/peptic ulcers, gastritis, gastrointestinal bleeding, glossitis, hematemesis, hepatitis, jaundice
- Hemic and Lymphatic System: ecchymosis, eosinophilia, leukopenia, melena, purpura, rectal bleeding, stomatitis, thrombocytopenia
- Metabolic and Nutritional: weight changes
- Nervous System: anxiety, asthenia, confusion, depression, dream abnormalities, drowsiness, insomnia, malaise, nervousness, paresthesia, somnolence, tremors, vertigo
- Respiratory System: asthma, dyspnea
- Skin and Appendages: alopecia, photosensitivity, sweating increased

CONTRAINDICATIONS

Diclofenac potassium is contraindicated in patients with known hypersensitivity to diclofenac. Diclofenac potassium should not be given to patients who have experienced asthma, urticaria, or other allergic-type reactions after taking aspirin or other NSAIDs. Severe, rarely fatal, anaphylactic-like reactions to NSAIDs have been reported in such patients

CHAPTER- IX

EXCIPIENT PROFILE

PHOSPHATIDYLCHOLINE (PHOSPHOLIPON 90)

CHEMICAL NAME [69]

[(2R)-3-hexadecanoyloxy-2-[(Z)-octadec-9-enoyl]oxypropyl] 2-(trimethyl azaniumyl)ethyl phosphat

EMPIRICAL FORMULA

$C_{42}H_{82}NO_8P$

MOLECULAR WEIGHT

790

DESCRIPTION

Pale yellow to yellow granular powder

SOURCE

Phosphatidylcholine occurs in all cellular organisms, being one of the major components of the phospholipid portion of the cell membrane.

PROPERTIES

- Moisture: maximum 1.5%
- Acid value: maximum 0.5
- Peroxide value: 0.5

FUNCTIONAL CATEGORY:

- Solubilizer
- Emulsifier
- Micelle forming agent

STORAGE

- Under dry condition, at maximum 8^{o}C sealed under inert gas.
- Stored in a freezer at -20^{o}C further improves the shelf life and is therefore recommendable.

HANDLING PRECAUTIONS

Eye protection and Gloves are recommended.

APPLICATIONS

- Preparation of mixed micelles, liposomes and microemulsions.
- Solubilizer for parenteral administration forms
- Emulsifier for pharmacy, dermatology and cosmetics

CHAPTER- X

EXPERIMENTAL DETAILS

STANDARD CURVE FOR DICLOFENAC POTASSIUM

Preparation of calibration medium

Phosphate buffer saline P^H: 7.4

2.38g of disodium hydrogen phosphate, 0.19g of potassium dihydrogen phosphate and 8g of sodium chloride is dissolved in sufficient water to produce 1000 ml. The P^H is adjusted if necessary.

Standard curve for Diclofenac Potassium [58]

100mg of drug is taken in a 100ml standard flask and dissolved in a small amount of phosphate buffer saline P^H: 7.4. Finally the solution is made up to 100ml with the same medium.

10ml of the above solution is pipetted out into another 100ml standard flask and make up to 100ml with phosphate buffer saline P^H: 7.4. Then from the above solution 5, 10, 15, 20, 25, ...50ml solutions are pipetted out into 10 different 100ml standard flasks and make up to 100ml with phosphate buffer saline P^H: 7.4 to get different concentrated solutions.

The drug in phosphate buffer saline P^H: 7.4, 10µg/ml is scanned to find the λmax in the UV-Visible spectrophotometer using phosphate buffer saline P^H: 7.4 as blank. The standard curve is plotted by taking concentration in X-axis and absorbance in Y- axis measured at λmax (276 nm).

FORMULATION OF DICLOFENAC POTASSIUM ETHOSOMES [57]

Various Diclofenac Potassium ethosomal formulations are prepared using 1-4% of phospholipon 90 and 10 to 40% of ethanol by classic mechanical dispersion method. The drug concentration kept as constant for each formulation 10mg/ml or 1% w/v. The composition of various ethosomal formulations represented in table no: 1

Preparation of ethosomes [57]

Classic mechanical dispersion method is followed to develop the ethosomes. Accurately weighed quantity of phospholipon 90 is dissolved in 10ml of chloroform: methanol (3:1) mixture in a clean, dry 250ml round bottom flask followed by removal of the organic solvents using rotary flash evaporator at 60rpm to form a thin lipid film in the flask. The traces of organic solvents mixture is further removed by maintaining the temperature and reduced pressure for additional 30 minutes with rotation after the thin film is formed.

Then the lipid film is hydrated with different concentration of hydroethanolic mixture containing Diclofenac Potassium (1% w/v) by rotation (60rpm, 1 hour) at room temperature. The preparation is vortexed followed by sonication at $4^{o}C$ in an ice bath using probe sonicator at 40W in three cycles of 5 minutes with 5 minutes rest in between the cycles. After sonication the ethosomal formulation is stored at $4^{o}C$ in refrigerator.

EVALUATION OF ETHOSOMES

The ethosomal formulations are evaluated for entrapment efficiency and invitro release studies through synthetic membrane in phosphate buffer saline P^{H}: 7.4.

Determination of entrapment efficiency [51, 52, 53, 63]

Entrapment efficiency of Diclofenac Potassium ethosomal vesicles is determined by centrifugation method. The vesicles are separated in a high speed cooling centrifuge at 20,000rom for 90 minutes in temperature maintained at 4^oC. The sediment and supernatant liquids are separated; amount of drug in the sediment is determined by lysing the vesicle using methanol. From this the entrapment efficiency is determined by the following equation,

Entrapment efficiency = D_E / D_T

Where,

D_E — Amount of drug in the ethosomal sediment

D_T— Theoretical amount of drug used to prepare the formulation (equal to amount of drug in supernatant liquid and in the sediment)

Invitro release studies [56, 62]

Invitro drug release is determined by using Franz diffusion cell. The synthetic membrane, presoaked in distilled water is mounted between the donor and receptor compartment. The receptor compartment is filled with phosphate buffer saline P^H: 7.4. The temperature is maintained at 32 ± 1^oC and the receptor compartment is constantly stirred by a magnetic stirrer at 100rpm. The ethosomal formulation is taken in donor compartment and the donor compartment is covered with aluminium foil to avoid any evaporation process.

Samples (0.5ml) are withdrawn through the sampling port of the diffusion cell at predetermined time intervals over 12 hours analyzed for drug content after suitable

dilution, using UV-Visible spectrophotometer at 276nm using phosphate buffer saline P^H:7.4 as blank. The receptor phase is immediately replenished with equal volume of fresh medium. Sink condition is maintained throughout the experiment. Triplicate experiments are conducted for each study.

The percentage of drug release is plotted against time to find the drug release pattern of all ethosomal formulations.

Formulation and evaluation of Diclofenac Potassium liposomes [57]

Preparation of liposomes

Phospolipon 90 - 4% w/v

Drug - 1% w/v

Liposomes containing 4% phospolipid is prepared by the same method of preparation of ethosomes that is classic mechanical dispersion method.

Phospholipon 90 is dissolved in 10ml of chloroform: methanol 3:1 mixture in a clean, dry 250ml round bottom flask followed by removal of organic solvents using rotary flash evaporator at 55^{o}C to form a thin lipid film on the wall of the flask. After removal of solvent traces, thin lipid film is hydrated with 1% w/v of drug in distilled water at 60rpm revolution for 1 hour at room temperature followed by sonication as described in ethosomal preparation.

Determination of entrapment efficiency of liposomes [55, 57]

Entrapment efficiency is determined like ethosomes by centrifugation method using cooling centrifuge at 20,000rpm for 90 minutes at in temperature maintained at 4^{o}C.

Amount of drug in the sediment is determined and the entrapment efficiency calculated like ethosomal formulations.

Comparison of invitro skin permeation of drug form various formulations [54, 57]

Invitro skin permeation of Diclofenac Potassium ethosomes, liposomes, drug hydroethanolic solution (1%w/v) and drug solution of phosphate buffer saline P^H: 7.4 (1%w/v) are studied using locally fabricated Franz diffusion cell with an effective permeation area and the receptor cell volume 2.54cm^2 and 17ml respectively.

Rats (male albino) 6 to 8 weeks old, weighing 120 to 150g are sacrificed for abdominal skin. After removing the hair the abdominal skin is separated from the underlying connective tissue with scalpel. The excised skin is placed on aluminium foil and the dermal side of the skin is gently teased off for any adhering fat and/or subcutaneous tissue. The skin is checked carefully to ensure the skin samples are free from any surface irregularity such as fine holes or cervices in the portion that is used for transdermal permeation studies. The in-vitro study was approved by the institutional ethical committee.

The skin is mounted between donor and receptor compartment with the stratum corneum side facing upward into the donor compartment. Phosphate buffer saline P^H: 7.4 is filled in the receptor compartment. The ethosomal formulation (500µl) is applied on the skin in donor compartment which is then covered with aluminium foil to avoid any evaporation process. Samples (0.5ml) are withdrawn through the sampling port of diffusion cell at predetermined time intervals over 12 hours, diluted to 10 ml with phosphate buffer saline P^H: 7.4 and analyzed for drug content by UV-Visible spectrophotometer at 276nm using phosphate buffer saline P^H: 7.4 as blank. The receptor medium is immediately replenished with equal volume of fresh medium.

Similar experiments are performed with diclofenac potassium liposomes, 1% drug dispersed in 40% hydroethanolic solution and 1% drug dispersed in phosphate buffer saline P^H: 7.4. Triplicate experiments are conducted for each study. Sink conditions are maintained in all the experiment. The percentage of drug release is plotted against time to find the drug release pattern.

Skin retention studies [51, 52]

The amount of Diclofenac Potassium retained in the skin is determined at the end of the 12 hours invitro permeation studies. The formulation remain in the invitro permeation experiment is removed by washing with distilled water. The receptor content is completely removed and replaced by 50% v/v ethanol in distilled water and kept for further 12 hours with stirring, sample is withdrawn from sampling port, diluted with phosphate buffer saline P^H: 7.4 and the absorbance is measured in a UV-Visible spectrophotometer at 276nm using phosphate buffer saline P^H: 7.4 as blank. This receiver solution diffused through the skin, disrupting any liposome and ethosome structure and extracting deposited drug from the skin, Thus giving a measure of skin deposition. Each formulation is investigated in 3 times.

Visualization by scanning electron microscopy (SEM) [57]

Scanning electron microscopy observes the size and shape of vesicles. One drop of ethosomal suspension of F16 formulation is mounted on a clear glass stub. It is air dried and gold coated using sodium auro thiomalate and visualized under scanning electron microscope at 10,000 magnifications.

Vesicular size and size distribution [51, 57, 62]

Dynamic light scattering technique is used to determine the vesicular size and size distribution. One drop of ethosomal formulation is diluted to 10ml with hydroethanolic mixture of the same proportion of the formulation and the measurements are taken. The size distribution of the liposome formulation is also determined after diluted with distilled water.

FT-IR studies

The interaction between ethosomal membrane component phospolipon 90 with drug is studied by using IR-Spectrophotometer.

Stability studies [51, 63]

The ethosomal formulations are stored in two different temperatures 4±2^{o}C, 25±2^{o}C and the entrapment efficiency is estimated for every 15 days.

Formulation of ethosomal gel [56]

The ethosomal formulation (F16) showing higher entrapment efficiency and lower particle size is incorporated in suitable gel base. The gel base is prepared using the following formula.

Carbopol 980 NF – 2g

Triehanolamine - 1.65ml

Purified water - 100 ml

Carbopol 980 NF is weighed and dispersed in 100ml warm purified water by constant stirring. The tri ethanolamine is added and stirred until a viscous smooth gel is formed.

Preparation of ethosomal gel [56]

The selected ethosomal formulation (F16) 30ml is centrifuged at $4^{o}C$, 20,000rpm for 90 minutes to seperate the ethosomal vesicles. The Ethosomal sediment which contains only the entrapped drug is collected, the entrapment efficiency is determined in10ml. The ethosomal sediment is incorporated into 10g of gel base to obtain the ethosomal gel formulation, contains approximately 1% w/w of drug.

Drug content of gel formulation

1g of gel formulation which contains approximately 10mg of drug is accurately weighed and transferred to 100ml standard flask. 10ml of mehtanol is added to lyse the ethosomal vesicles and shaken well. The volume is made up to 100ml with distilled water.

10ml of the above solution is pipetted out into 100ml standard flask and the volume is made up to 100ml with distilled water. The absorbance is measured at 276 in UV-Visible spectrophotometer using distilled water as blank.

Pharmacodynamic studies [54]

The anti inflammatory activity is carried out by carrageenan induced paw oedema method to compare the activity of marketed product and the formulated gel.

Materials and methods

Animals: Male albino rats of wister strain (150-200) are used for anti-inflammatory activity. The rats are fed with standard food and water. Food is withdrawn 12 hours before and during the experimental studies.

Anti inflammatory activity

The animals are divided into four groups having three animals in each group.

Group1: Control receiving normal saline

Group2: Control receiving gel base without drug

Group3: Receiving Diclofenac Potassium ethosomal gel

Group4: Receiving plain Diclofenac gel (marketed)

After application of the samples for all the animals, 0.1ml of 1% carrageenan in normal saline is injected. Then the paw oedema is measured by using plethysmometer at 0, 1, 2, 3 and 4 hours. Mean paw oedema is measured and percentage of inhibition is calculated. The in-vivo study was approved by the institutional ethical committee.

CHAPTER- XI

RESULTS AND DISCUSSION

STANDARD CURVE FOR DICLOFENAC POTASSIUM [58]

The λ_{max} of Diclofenac Potassium was determined by scanning 10µg/ml drug in phosphate buffer saline P^H: 7.4 and it showed the λ_{max} at 276nm. The λ_{max} of Diclofenac Potassuim is showed as UV graph in figure 7.

Calibration curve of Diclofenac Potassium was plotted by measuring the absorbance of different concentrations of the drug in phosphate buffer saline P^H: 7.4 at 276 nm. The linear correlation co-efficient was obtained for calibration of Diclofenac Potassium in phosphate buffer saline P^H: 7.4. Diclofenac Potassuim obeys the beer's law within the concentration range of 5 to 50µg/ml. Calibration readings shown in table no: 2 and the calibration plot of Diclofenac Potassuim is shown in figure 8.

FORMULATION OF ETHOSOMES [57]

Ethosomal formulations were formulated by classic mechanical dispersion method as per the formula given in table no: 1 and gave translucent, uniform dispersion of ethosomal vesicles. The formation of ethosomal vesicle was confirmed by scanning electron microscopy shown in figure 9.

ENTRAPMENT EFFICIENCY

In ethosomal and liposomal formulations, the impact of phospholipid and ethanol concentration on entrapment efficiency was considerably significant. The range of entrapment efficiency of sixteen ethosomal formulations were observed about 18.74% to 72.91% and are shown in table no: 3 and figure no 10. The maximum entrapment

efficiency was obtained for the formulation prepared with 4% phospholipid and 40% ethanol. The entrapment efficiency of liposomes prepared from 4% w/v of phospholipid was 42.46±1.01. The greater entrapment of Diclofenac Potassuim in ethosomes than the conventional liposomes could be attributed to the greater retention of Diclofenac Potassuim in ethanol present in ethosomal core.

Effect of phospholipid on entrapment efficiency [51, 52, 53, 63]

Formulations F1, F5, F9 and F13 were prepared with 10%v/v ethanol and 1%, 2%, 3% and 4% w/v of phospholipid showed the entrapment efficiency of 18.74%, 27.77%, 39.18% and 55.35% respectively.

Formulations F2, F6, F10 and F14 were prepared with 20%v/v ethanol and 1%, 2%, 3% and 4% w/v of phospholipid showed the entrapment efficiency of 27.17%, 31.04%, 41.86% and 60.41% respectively.

Formulations F3, F7, F11 and F15 were prepared with 30%v/v ethanol and 1%, 2%, 3% and 4% w/v of phospholipid showed the entrapment efficiency of 30.94%, 37.07%, 55.33% and 64.47% respectively.

Formulations F4, F8, F12 and F16 were prepared with 40%v/v ethanol and 1%, 2%, 3% and 4% w/v of phospholipid showed the entrapment efficiency of 34.42%, 40.37%, 63.69% and 72.91% respectively.

Among them, the ethosomal formulations prepared with 4% phospholipid showed the higher entrapment efficiency than the other ethosomal formulations prepared with 1%, 2% and 3%w/v of phospholipids. The entrapment efficiency was increased in the order of **1% < 2% < 3% < 4%w/v** of phospholipid concentration.

The results revealed that the increasing concentration of phospholipid the percentage entrapment efficiency was increased.

Effect of ethanol on entrapment efficiency [56, 62]

Embodying high concentration of ethanol in liposomes led to the formation of ethosomes which are very potent transdermal drug delivery system, having range of molecules including lipophilic drugs and are effective at delivering drugs through the skin.

Formulations F1, F2, F3 and F4 were prepared with 1%w/v of phospholipid and 10%, 20%, 30% and 40% v/v of ethanol showed the entrapment efficiency of 18.74%, 27.17%, 30.94% and 34.42% respectively.

Formulations F5, F6, F7 and F8 were prepared with 2%w/v of phospholipid and 10%, 20%, 30% and 40% v/v of ethanol showed the entrapment efficiency of 27.77%, 31.04%, 37.07% and 40.37% respectively.

Formulations F9, F10, F11 and F12 were prepared with 3%w/v of phospholipid and 10%, 20%, 30% and 40% v/v of ethanol showed the entrapment efficiency of 39.18%, 41.86%, 55.33% and 63.69% respectively.

Formulations F13, F14, F15 and F16 were prepared with 4%w/v of phospholipid and 10%, 20%, 30% and 40% v/v of ethanol showed the entrapment efficiency of 55.35%, 60.41%, 64.47% and 72.91% respectively.

The entrapment efficiency was increased in the order of **10% < 20% < 30% < 40%v/v** of ethanol concentration. The results revealed that the increasing the concentration of ethanol the percentage entrapment efficiency was increased. Ethanol may exert a stabilizing effect in the formulation, preventing or at least delaying the

formation of vesicle aggregates, because of electrostatic repulsions. The factor that governing the stability and hence the entrapment efficiency of vesicles is the vesicles containing high ethanol concentrations have thinner membranes, corresponding to the formation of the formation of a phase with interpenetrating hydrocarbon chains.

INVITRO RELEASE STUDIES

The invitro release studies of Diclofenac Potassuim ethosomes was done by using Franz diffusion cell and phosphate buffer saline P^H: 7.4 as medium. The time dependent permeation profile of ethosomal formulations are given in table no: 4A to 4D and figure 11-18.

RELEASE BEHAVIOUR OF DICLOFENAC POTASSIUM IN PBS P^H: 7.4

Effect of phospholipid concentration on drug release [61]

Ethosomes are not able to improve skin delivery of non-entrapped drug. The drug molecule should be incorporated in ethosomal vesicles for optimum delivery under non occlusive conditions. The entrapment efficiency was increased with increase in phospholipon 90 concentrations and the non-entrapped drug concentration might be decreased. Thus decrease in the concentration of unentrapped drug and increase in concentration of entrapped drug caused enhancement of drug release with increasing concentration of phospholipid.

Formulations F1, F5, F9 and F13 were prepared with ethanol concentration of 10%v/v and 1%, 2%, 3% and 4% w/v of phospholipid showed the cumulative percentage of drug release 6.63%, 21.87%, 45.11% and 57.22% respectively at the end of 12 hours study.

Formulations F2, F6, F10 and F14 were prepared with ethanol concentration of 20%v/v and 1%, 2%, 3% and 4% w/v of phospholipid showed the cumulative percentage of drug release 10.22%, 25.20%, 50.32% and 61.99% respectively at the end of 12 hours study.

Formulations F3, F7, F11 and F15 were prepared with ethanol concentration of 30%v/v and 1%, 2%, 3% and 4% w/v of phospholipid showed the cumulative percentage of drug release 12.85%, 38.52%, 58.17% and 68.60% respectively at the end of 12 hours study.

Formulations F4, F8, F12 and F16 were prepared with ethanol concentration of 40%v/v and 1%, 2%, 3% and 4% w/v of phospholipid showed the cumulative percentage of drug release 16.38%, 50.86%, 63.15% and 70.96% respectively at the end of 12 hours study.

Among them, the ethosomal formulations prepared with 4% phospholipid showed the higher cumulative percentage of drug release than the other ethosomal formulations prepared with 1%, 2% and 3%w/v of phospholipids.

The cumulative percentage of drug release was increased in the order of **1% < 2% < 3% < 4%w/v** of phospholipid concentration. The results of the phospholipid concentration on drug release were shown in table no: 4A to 4D and figure 11-14.

Effect of ethanol concentration on drug release [55, 57, 58]

As increasing the concentration of ethanol in ethosomal formulation, increase the membrane fluidity of ethosomal vesicle, causes formation of deformable vesicles that can penetrate through small pores having much lesser size than the vesicles and are confirmed by the following results.

Formulations F1, F2, F3 and F4 were prepared by 1%w/v of phospholipon 90 with increasing concentration of ethanol 10%, 20%, 30% and 40%v/v showed the cumulative percentage of drug release 6.63%, 10.22%, 12.85% and 16.38% respectively at the end of 12 hours study.

Formulations F5, F6, F7 and F8 were prepared by 2%w/v of phospholipon 90 with increasing concentration of ethanol 10%, 20%, 30% and 40%v/v showed the cumulative percentage of drug release 21.87%, 25.20%, 38.52% and 50.86% respectively at the end of 12 hours study.

Formulations F9, F10, F11 and F12 were prepared by 3%w/v of phospholipon 90 with increasing concentration of ethanol 10%, 20%, 30% and 40%v/v showed the cumulative percentage of drug release 45.11%, 50.32%, 58.17% and 63.15% respectively at the end of 12 hours study.

Formulations F13, F14, F15 and F16 were prepared by 4%w/v of phospholipon 90 with increasing concentration of ethanol 10%, 20%, 30% and 40%v/v showed the cumulative percentage of drug release 57.22%, 61.99%, 68.60% and 70.76% respectively at the end of 12 hours study.

Among them, the ethosomal formulations prepared with 40%v/v ethanol showed the higher cumulative percentage of drug release than the other ethosomal formulations prepared with 10%, 20% and 30%v/v of ethanol concentrations.

The cumulative percentage of drug release was increased in the order of **10% < 20% < 30% < 40%v/v** of ethanol concentration. The results of the ethanol concentration on drug release were shown in table no: 4A to 4D and figure 15-18.

FORMULATION AND EVLUATION OF LIPOSOMES [56]

Liposomal formulation was formulated by classic mechanical dispersion method with 4%w/v of phospholipon 90 without alcohol. The liposomal formulation was translucent, uniform dispersion of vesicles and showed the percentage entrapment efficiency of 42.46±1.01%.The liposomal vesicles showed larger mean vesicle size of 422±38nm, than the ethosomal formulations shown in figure 19.

COMPARISON OF INVITRO SKIN PERMEATION OF DRUG FROM VARIOUS FORMULATIONS [56]

The skin permeation of Diclofenac Potassuim from ethosomal system, liposomal system, hydroethanolic and phosphate buffer saline P^H: 7.4 drug solutions through the rat skin were measured in Franz diffusion cell for a period of 12 hours. Among this ethosomal system showed highest drug release than the other formulations. The cumulative percentage of drug release from ethosomal system, liposomal system, hydroethanolic and phosphate buffer saline P^H: 7.4 drug solutions were 60.37%,15.95%,49.90% and 11.65% respectively at the end of 12 hours study shown in table no: 5 figure 20.

Ethosomal system showed 4 times and 5 times higher cumulative percentage of drug release than the liposomal and phosphate buffer saline P^H: 7.4 solution respectively. The permeation enhancement of drug from ethosomes was much greater than the hydroethanolic solution.

The stratum corneum lipid multilayer's are densely packed and highly conformationally ordered at physiological temperature. Ethanol interacts with lipid molecules in the polar head group region, resulting in a reduction in the phase transition

temperature T_m of stratum corneum lipids, increasing their fluidity. The intercalation of ethanol into the polar head group environment can result in an increase in the membrane permeability. In addition to the effects of ethanol on stratum corneum structure, the ethosome itself may interact with the stratum corneum barrier. Ethanol may also provide the vesicles with softness and flexibility that allows them to more easily penetrate into deeper layers of the skin and the ethosome vesicles can forge paths in the stratum corneum. The release of drug in the deep layers of the skin and its transdermal absorption could then be result of fusion of ethosomes with skin lipids and drug release at various points along the penetration pathway.

SKIN RETENSION STUDY [57]

The Ethosomal formulation,liposomal formulation, hydroethanolic solution and phosphate buffer saline P^H: 7.4 drug solutions showed the skin deposition 619.6±18.3µg/cm^2, 363.16±7µg/cm^2, 220.86±14.2µg/cm^2 and 136.46±5.7µg/cm^2 respectively at the end of 12 hour experiment shown in table no: 6 and figure:21. The skin deposition of ethosomal formulation was approximately 2, 3 times and 5 times greater than the hydroethanolic solution, liposomal formulation and phosphate buffer saline P^H: 7.4 drug solution respectively. The ethosomes showed significantly higher skin deposition possibly due to combined effect of ethanol and phospholipid thus providing a mode for dermal and transdermal delivery of Diclofenac Potassuim.

VISUALISATION BY SCANNING ELECTRON MICROSCOPY (SEM)

The analysis of the ethosomal formulation by SEM confirmed the three dimensional nature of ethosomes, justifying the vesicular characteristics possessed by this novel carrier shown in figure 9.

VESICULAR SIZE AND SIZE DISTRIBUTION

The effect of phospholipid and ethanol concentration on the size of the ethosome vesicles was investigated using Dynamic Light Scattering technique.

Effect of phospholipid concentration on vesicular size [57]

Formulations F1, F5, F9 and F13 were prepared with 10% ethanol concentration and 1%, 2%, 3% and 4%w/v of phospholipon 90 respectively. F1, F5, F9 and F13 formulations had the mean particle size of 125±20nm, 137±32nm, 272±21nm and 325±37nm respectively shown in table no: 7 and figure 19. As increasing the concentration of phospholipid, the mean vesicular size was increased in the ethosomal formulations. The mean vesicular size was increased in the order of **1% < 2% < 3% < 4%w/v** of phospholipid concentration.

Effect of ethanol concentration on vesicular size [57, 55]

Ethosomal formulations F13, F14, F15 and F16 were prepared by 4%w/v of phospholipon 90 with increasing concentration of ethanol 10%, 20%, 30% and 40% respectively. F13, F14, F15 and F16 formulations showed mean vesicular size 325±37nm, 301±32nm, 265±18nm and 251±23nm respectively.

The mean vesicular size was decreased in the order of **10% > 20% > 30% > 40%v/v** of ethanol concentration. As increasing the concentration of ethanol, the mean vesicular size was decreased in the ethosomal formulations. Liposomal formulation showed the larger vesicular size of 422±38nm than the ethosomal formulations prepared with 4%w/v of phospholipid shown in figure 19.

FT-IR STUDIES

The interaction between ethosomal membrane component and drug was determined by comparing the IR spectrum of the standard Diclofenac potassium and the physical mixture of Diclofenac potassium and phospholipon 90.

The results of IR studies revealed that no interaction between ethosomal membrane with drug. The results are shown in IR graphs.

STABILITY OF ETHOSOMES

Stability studies of ethosomal formulations were carried out by storing at 4^oC and $25^{o\pm}2^oC$ for 4 weeks. The retention of drug in the ethosomal formulations was calculated immediately after the preparation and was taken as 100% retention. The percentage of drug retention in the ethosomal preparation at 2 week time intervals was determined. The results are shown in table no: 8A and 8B.

The results showed that the drug retention capacity was more with ethosomal formulation stored at $4^o\pm2^oC$ than $25^o\pm2^oC$. Hence increase in temperature and storage period decreased the drug retention capacity.

FORMULATION OF ETHOSOMAL GEL

Drug content analysis

The separated vesicles of ethosomal formulation F16 which contains 4%w/v of phosphatidylcholine and 40%v/v of ethanol was incorporated in the carbopol gel base. The drug content of the prepared gel was 99.2±0.85 estimated spectrophotometrically at 276nm.

PHARMACODYNAMIC STUDIES [54]

The pharmacodynamic study was carried out to find out the anti inflammatory activity of ethosomal gel preparation and correlate it with marketed gel formulation. It was assessed by carrageenan induced paw oedema method in albino rats. After 4 hours ethosomal gel formulation showed marked percentage inhibition of inflamation (44.44%) when compared to marketed formulation (35.80%) with lesser time for onset of action. Hence, this ethosomal formulation may be considered as a good choice to improve absorption of the anti-inflammatory drug. The results of the anti-iflammatory study were shown in table no: 9A, 9B and figure 22.

CHAPTER- XII

SUMMARY AND CONCLUSION

- The purpose of this work was to characterize a novel ethosomal carrier containing Diclofenac Potassium and to investigate the delivery of Diclofenac Potassium from ethosomes.
- The percentage entrapment efficiency was increased with increasing concentration of phospholipid as well as ethanol. The results of entrapment efficiency revealed that the ethosomal formulation containing 4% w/v phospholipon 90 and 40%v/v ethanol F16 showed the highest entrapment efficiency than the other ethosomal formulations.
- The invitro release studies through synthetic membrane revealed that the increasing concentration of both phospholipid and ethanol not only increase the percentage entrapment deficiency but also the invitro release of drug. The ethosomal formulation containing 4%w/v phospholipon 90 and 40%v/v ethanol showed the higher cumulative percentage of drug release than the other ethosomal formulations.
- The comparison of invitro skin permeation of ethosomal, liposomal, hydroethanolic drug solution and phosphate buffer saline P^H: 7.4 drug solutions revealed that ethosomal formulation showed significantly higher cumulative percentage of drug permeation than the other formulations. The cumulative percentage of drug release was decreased in the order of **Ethosomal formulation > Hydroethanolic drug solution > Liposomal formulation > phosphate buffer saline P^H: 7.4 drug solution.**
- The amount of skin retention of various forulations decreased in the order of **Ethosomal formulation > Hydroethanolic drug solution > Liposomal formulation > phosphate buffer saline P^H: 7.4 drug solution.**

- The Scanning Electron Microscope photograph of the ethosomal formulation confirmed the three dimensional nature of ethosomes.
- The analysis of particle size revealed that increasing the phospholipid concentration increases the vesicular size. Comparatively liposomal vesicles had higher vesicular size than all the ethosomal formulations.
- The results of IR studies proved that no interaction between ethosomal membrane with drug.
- Stability studies indicated that the drug retention capacity of ethosomes was more in $4^{o}C$ than $25^{o}C$.
- The gel formulation containing Diclofenac Potassium ethosomes was easily prepared and found to be homogenous in composition.
- The pharmacodynamic study of ethosomal gel showed enhanced anti inflammatory activity when compared to marketed preparation. Therefore the present study suggests the potency of the ethosomes to enhance the therapeutic effect and minimize the side effects of Diclofenac Potassium.

From the above study it is concluded that the ethosomes can be considered as promising tool for transdermal drug delivery system which produces deeper penetration thereby enhances the therapeutic potential of drug molecules. This technique may be applied to prepare the ethosomal formulations containing anticancer drugs to treat the deep seated tumors, because ethosomes can penetrate deeply. Ethosomes can be applied to prepare the drug formulations that required acting systemically, since it can improve the systemic availability through the transdermal route. Further pharmacokinetic and clinical studies may be carried out in future.

FIGURE- 11
COMPARISON OF INVITRO OF ETHOSOMES CONTAINING 1% PHOSOPHOLIPID IN PHOSPHATE BUFFER SALINE pH: 7.4

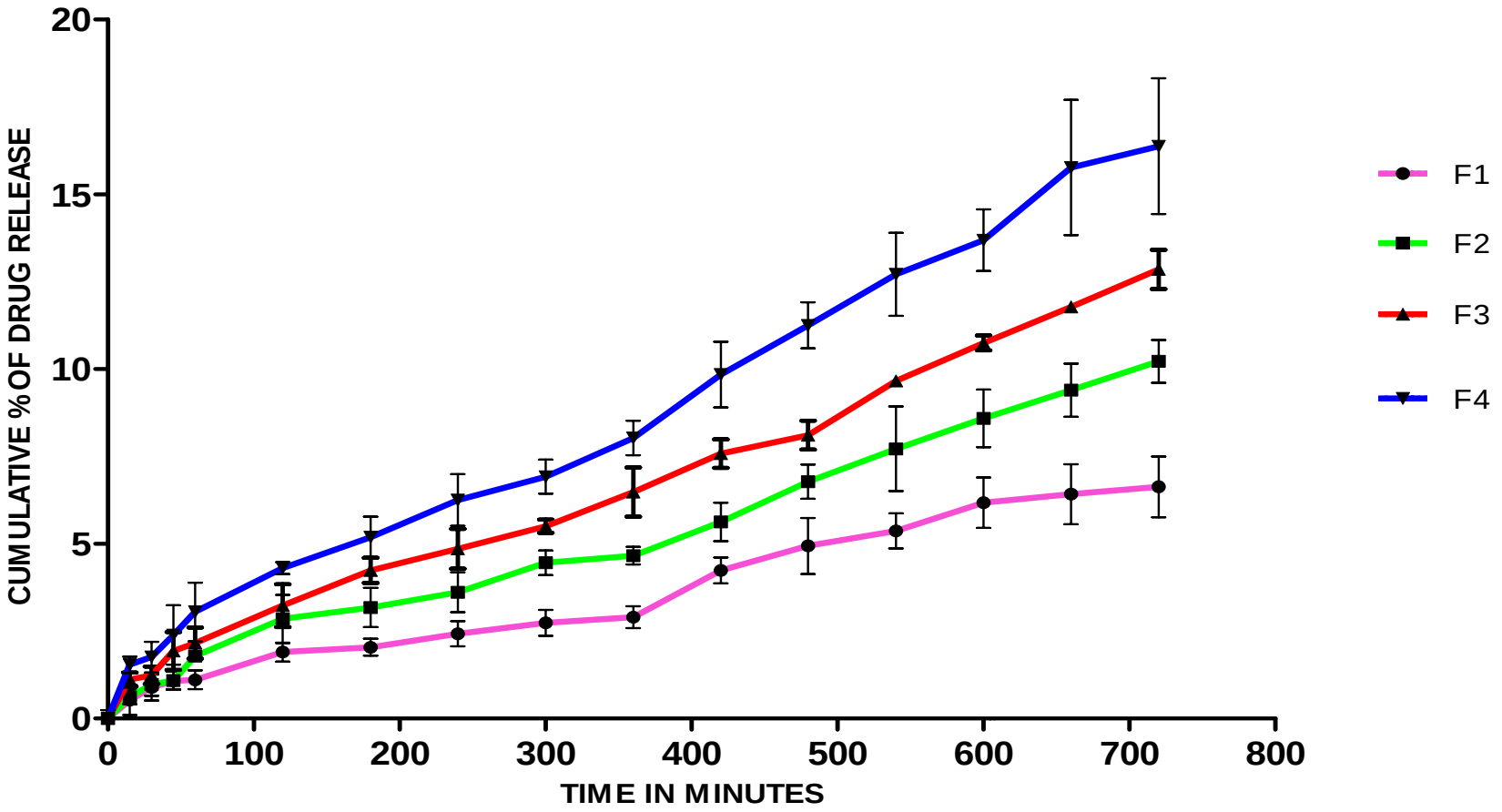


FIGURE- 12
COMPARISON OF INVITRO OF ETHOSOMES CONTAINING 2% PHOSOPHOLIPID IN PHOSPHATE BUFFER SALINE pH: 7.4

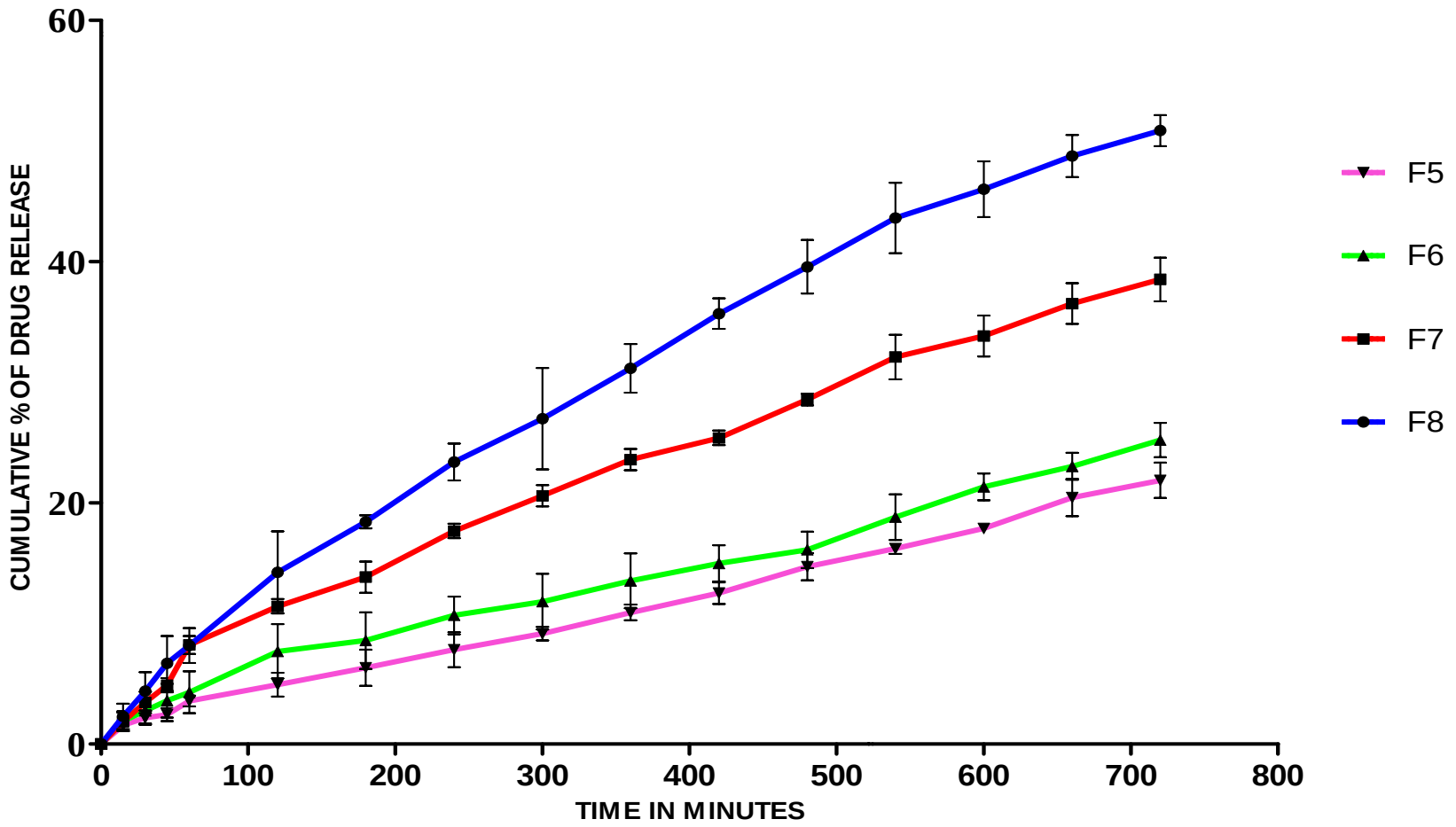


FIGURE- 13
COMPARISON OF INVITRO OF ETHOSOMES CONTAINING 3% PHOSOPHOLIPID IN PHOSPHATE BUFFER SALINE pH: 7.4

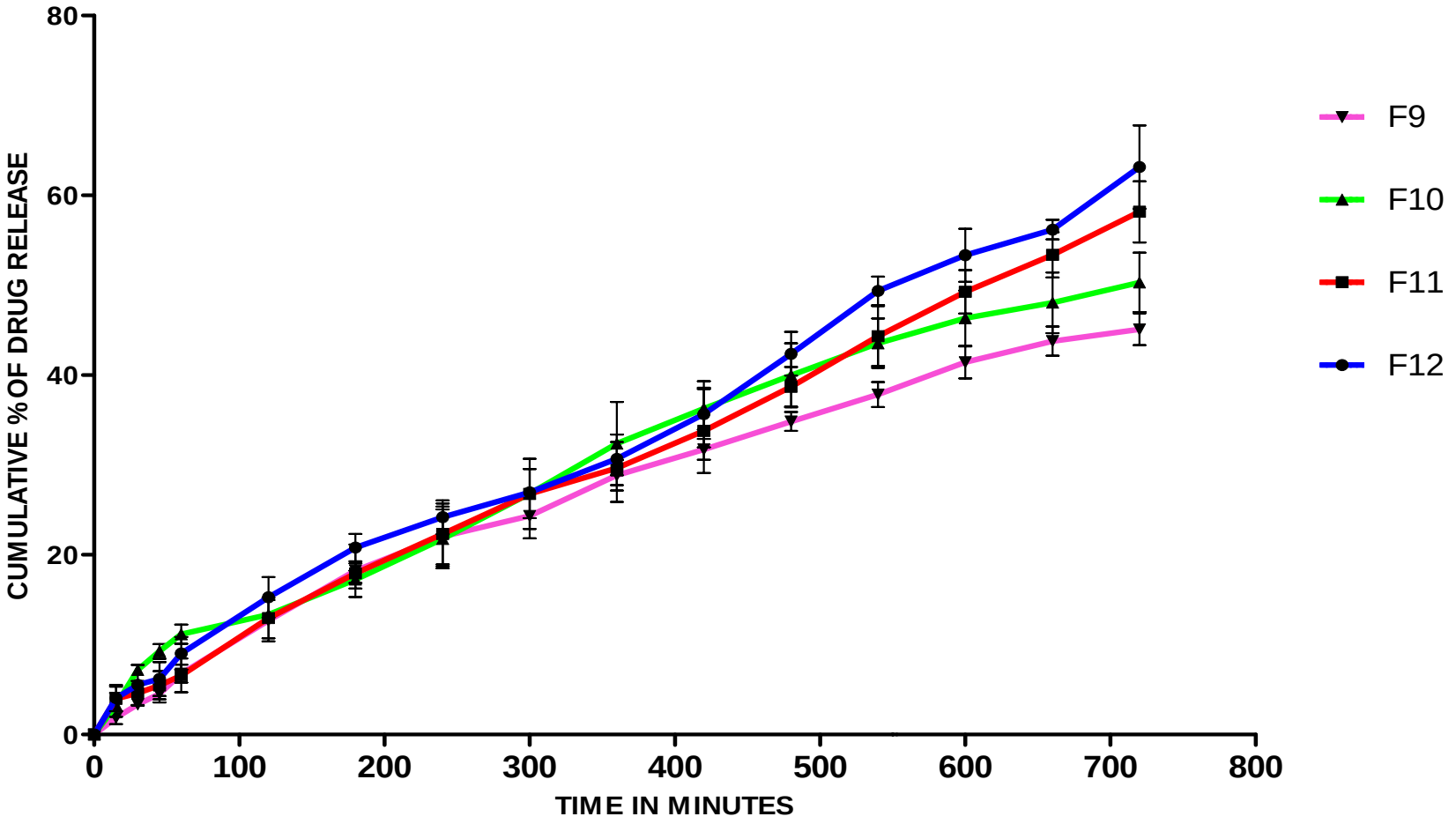


FIGURE- 14
COMPARISON OF INVITRO OF ETHOSOMES CONTAINING 4%W/V PHOSPHOLIPID IN PHOSPHATE BUFFER SALINE pH 7.4

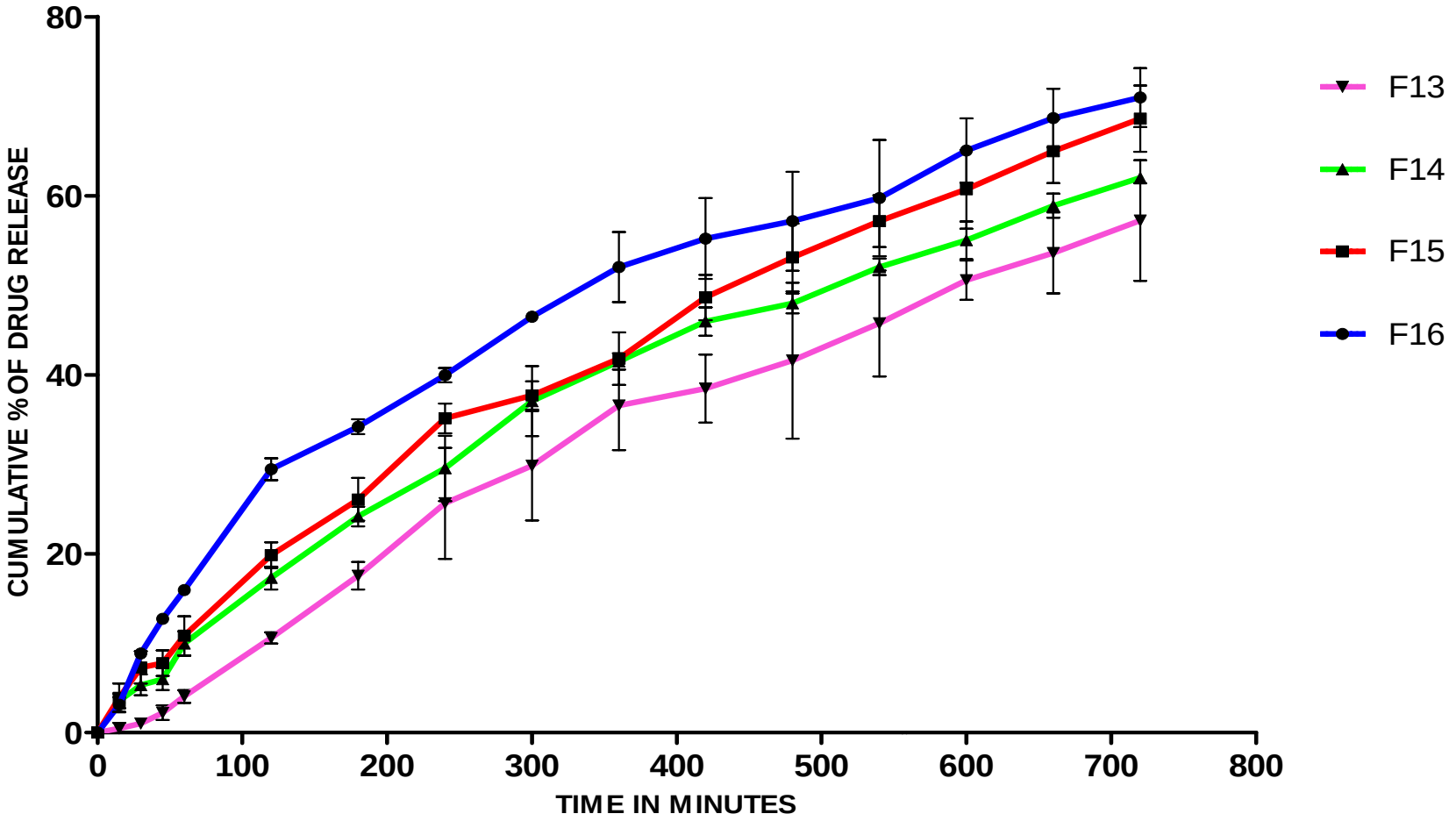


FIGURE- 15
COMPARISON OF INVITRO OF ETHOSOMES CONTAINING 10%V/V ETHANOL IN PHOSPHATE BUFFER SALINE pH 7.4

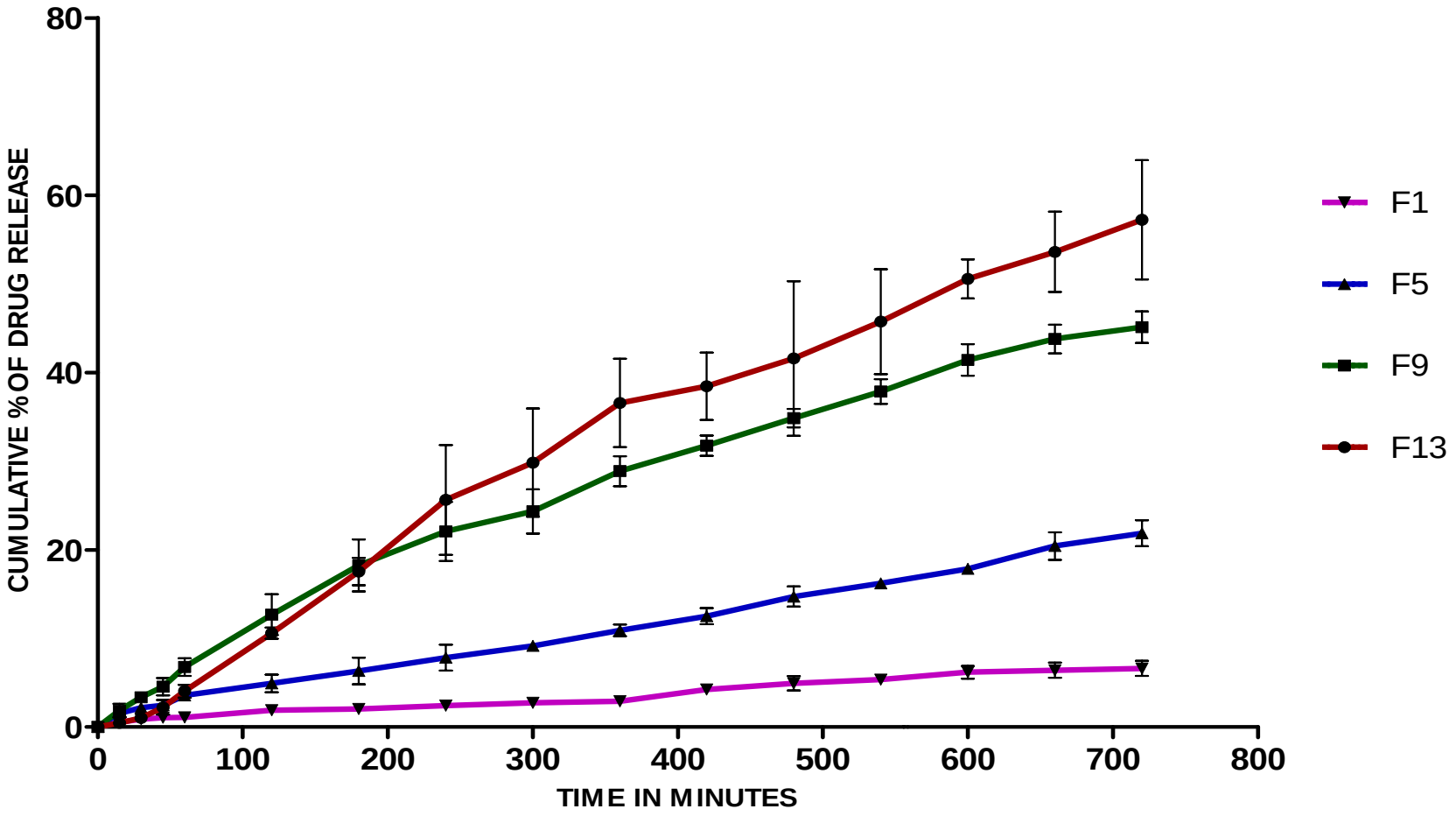


FIGURE- 16
COMPARISON OF INVITRO OF ETHOSOMES CONTAINING 20%V/V ETHANOL IN PHOSPHATE BUFFER SALINE pH 7.4

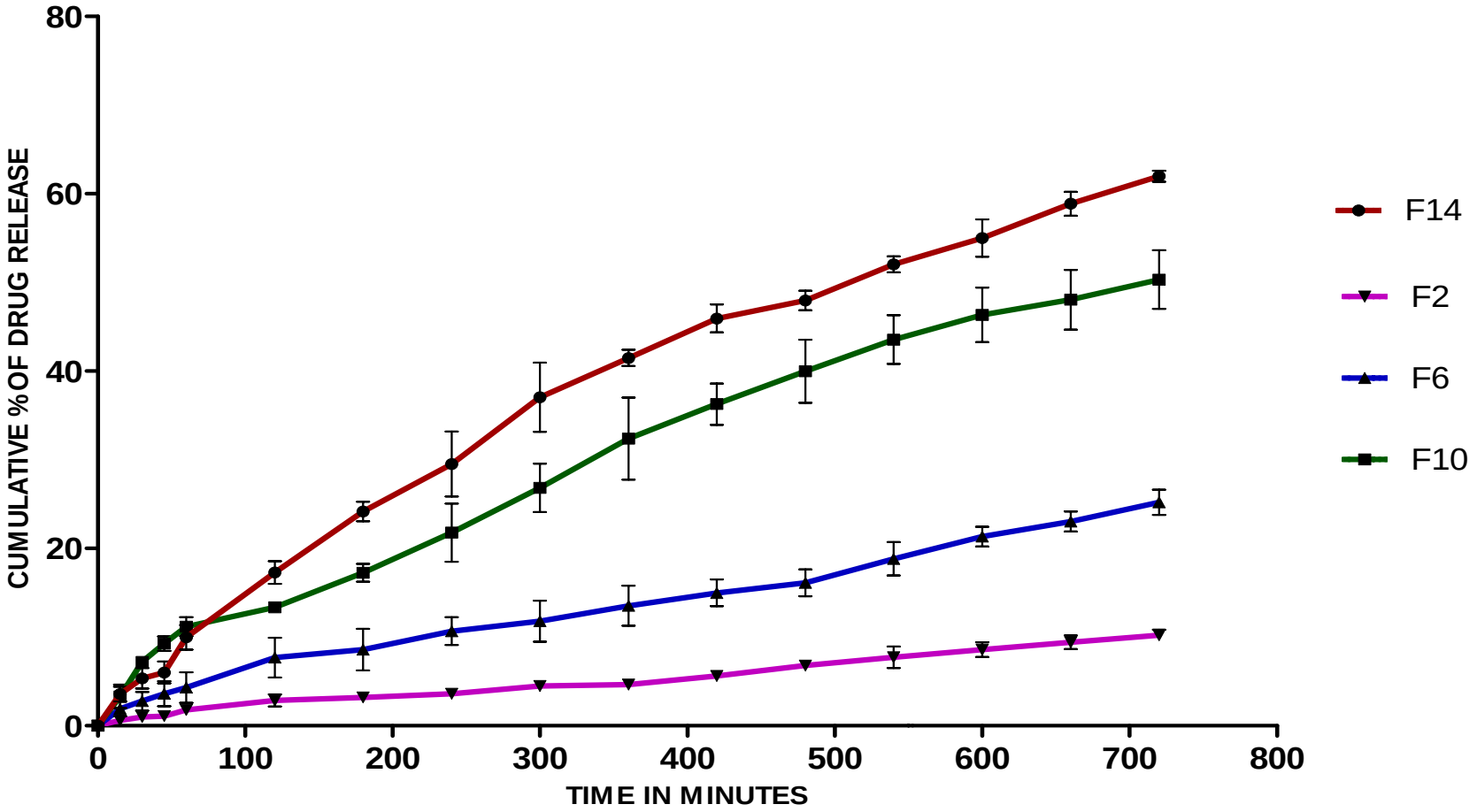


FIGURE- 17
COMPARISON OF INVITRO OF ETHOSOMES CONTAINING 30%V/V ETHANOL IN PHOSPHATE BUFFER SALINE pH 7.4

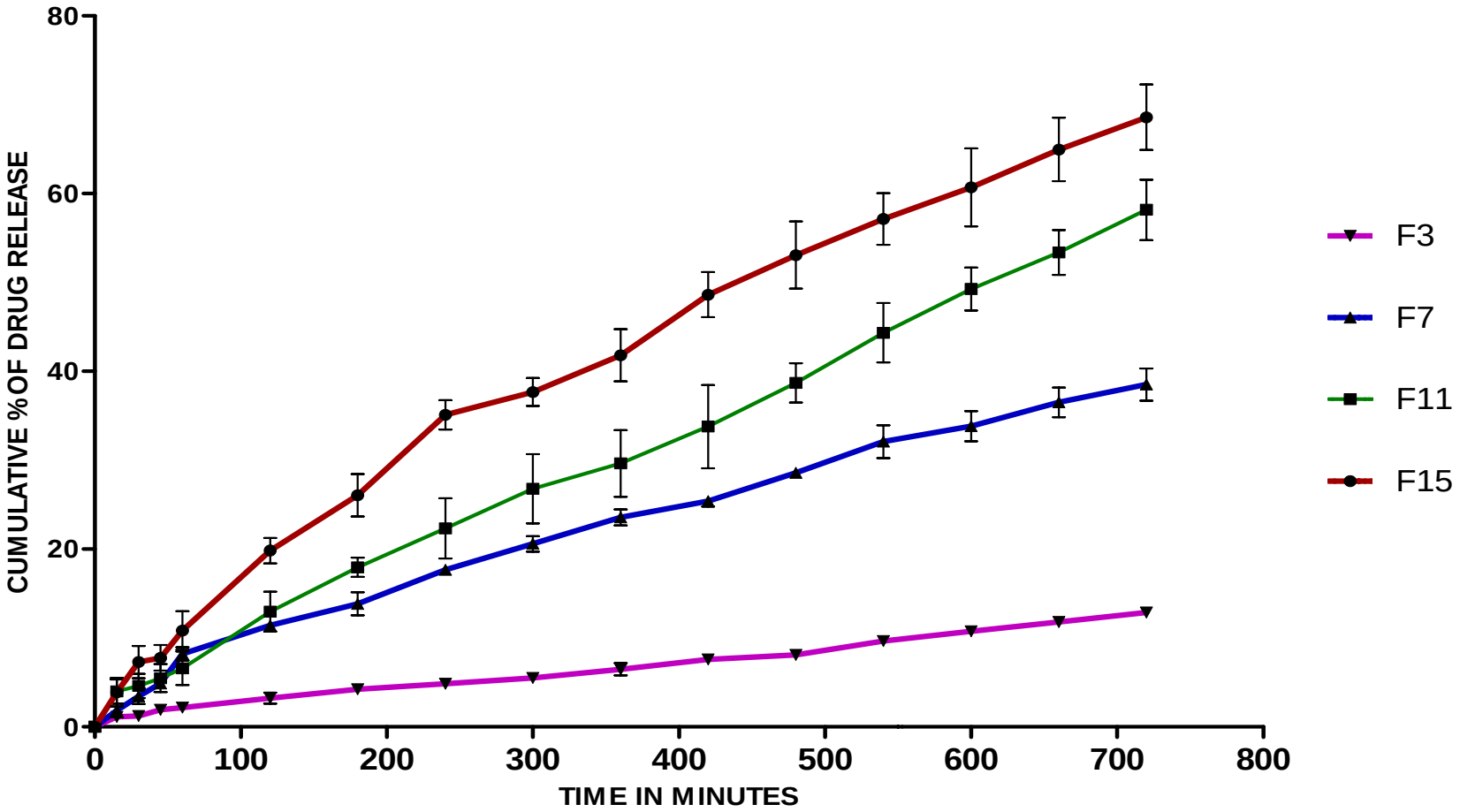


FIGURE- 18
COMPARISON OF INVITRO OF ETHOSOMES CONTAINING 40%V/V ETHANOL IN PHOSPHATE BUFFER SALINE pH: 7.4

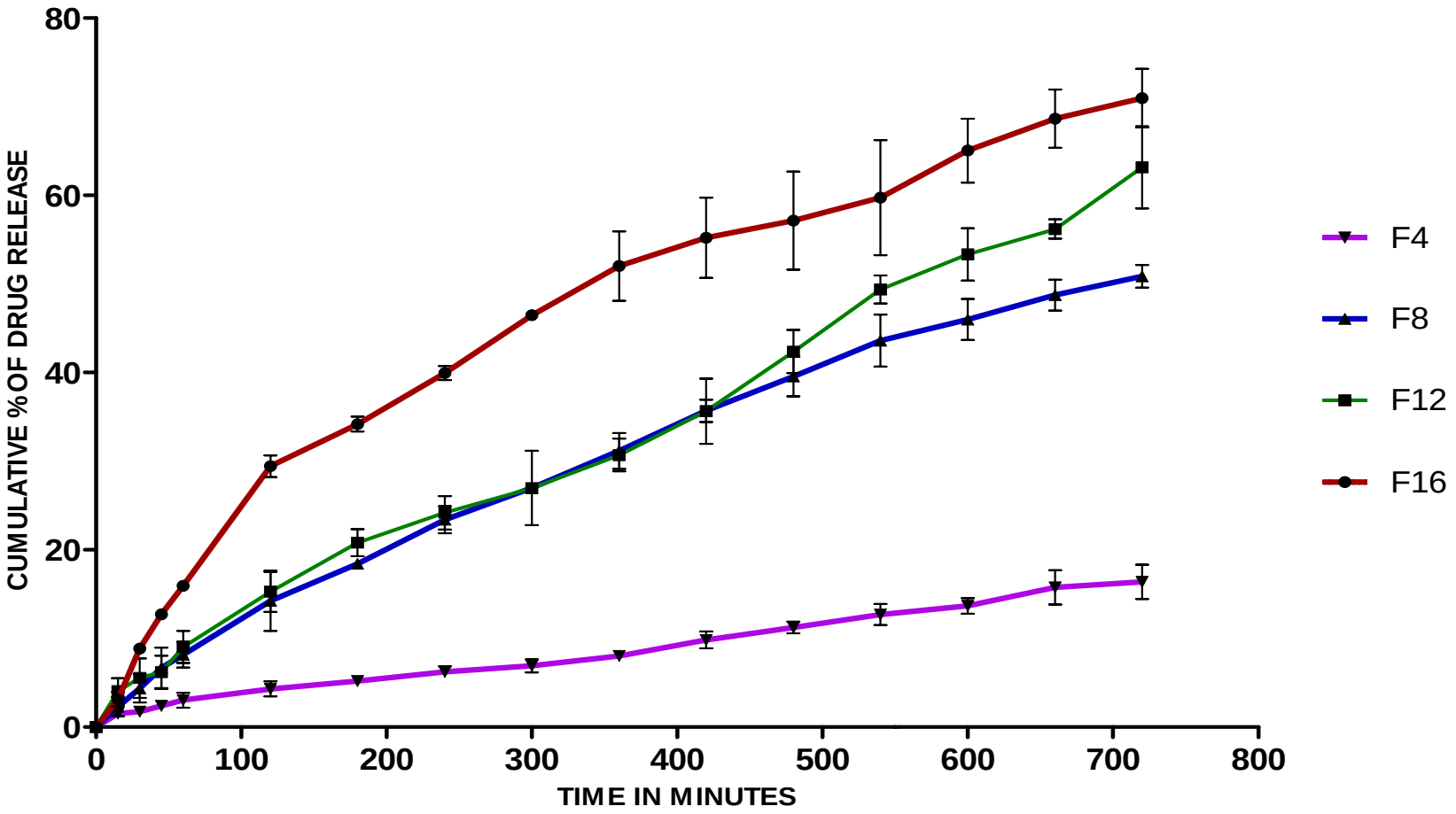


FIGURE- 20

COMPARISON OF INVITRO SKIN PERMEATION OF DRUG FROM VARIOUS FORMULATIONS IN PHOSPHATE BUFFER SALINE pH 7.4

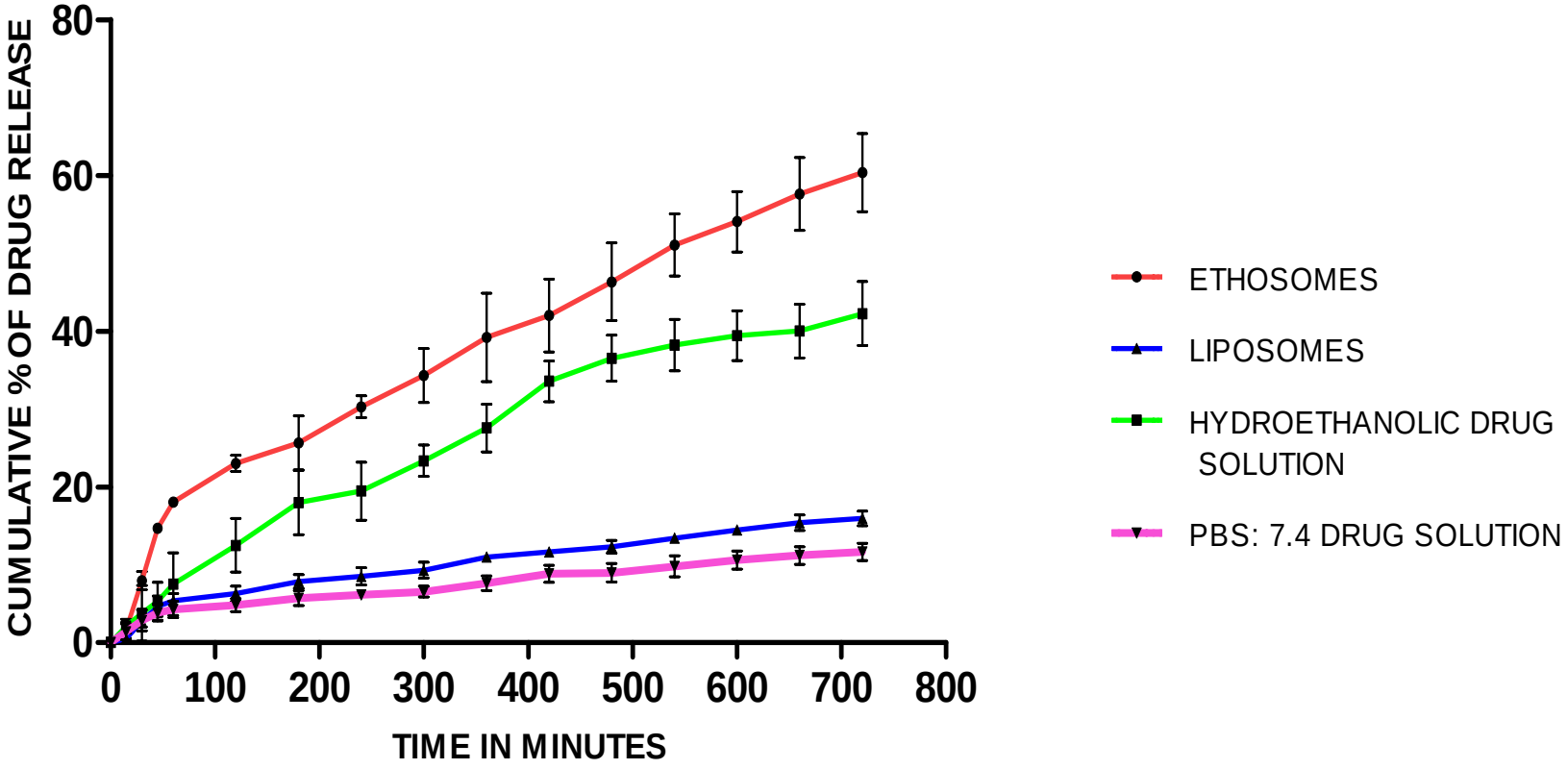


FIGURE- 8

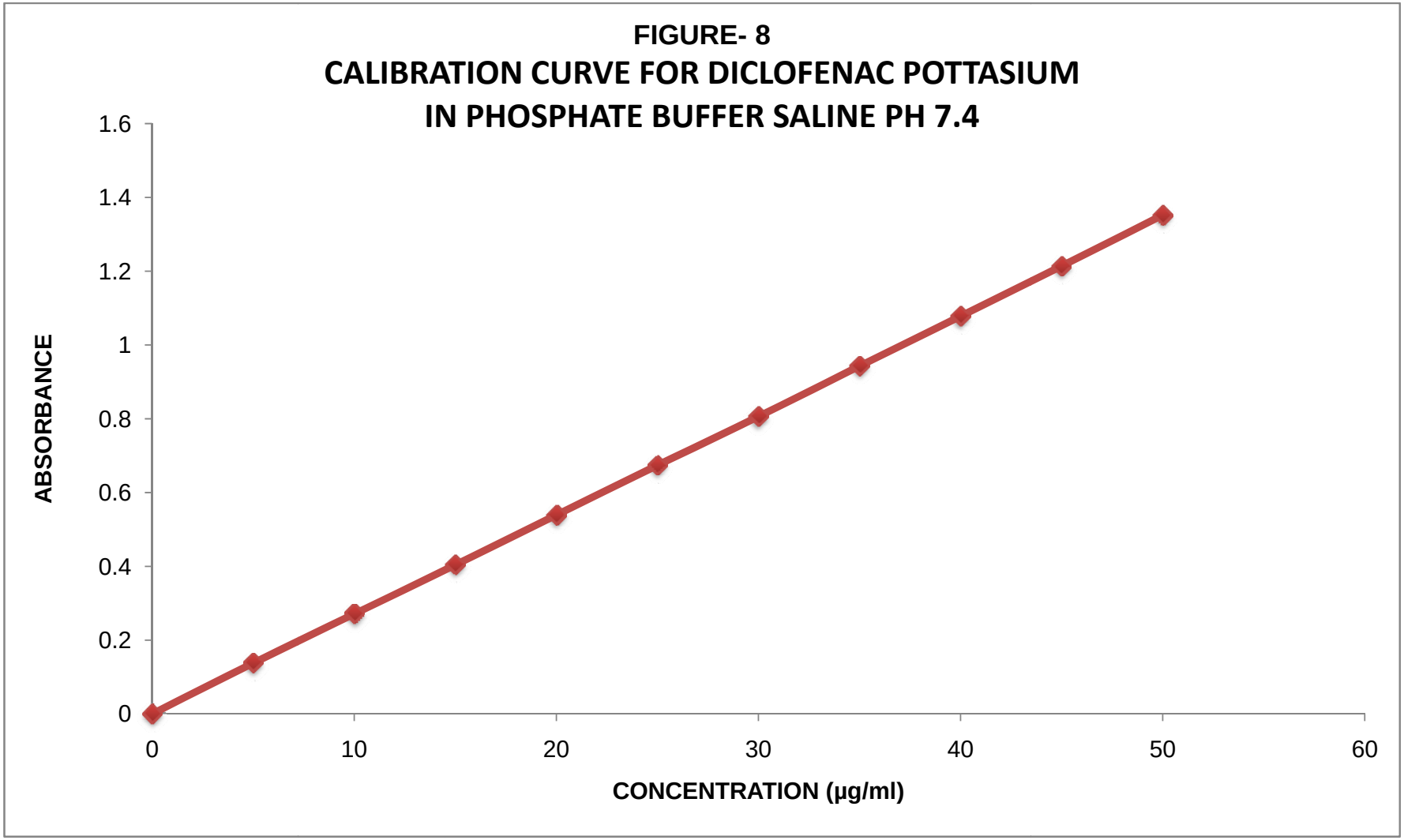

CALIBRATION CURVE FOR DICLOFENAC POTTASIUM
IN PHOSPHATE BUFFER SALINE PH 7.4
ABSORBANCE
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
10
20
30
40
50
60
CONCENTRATION (µg/ml)

FIGURE- 10
ENTRAPMENT EFFICIENCY OF ETHOSOMAL FORMULATIONS

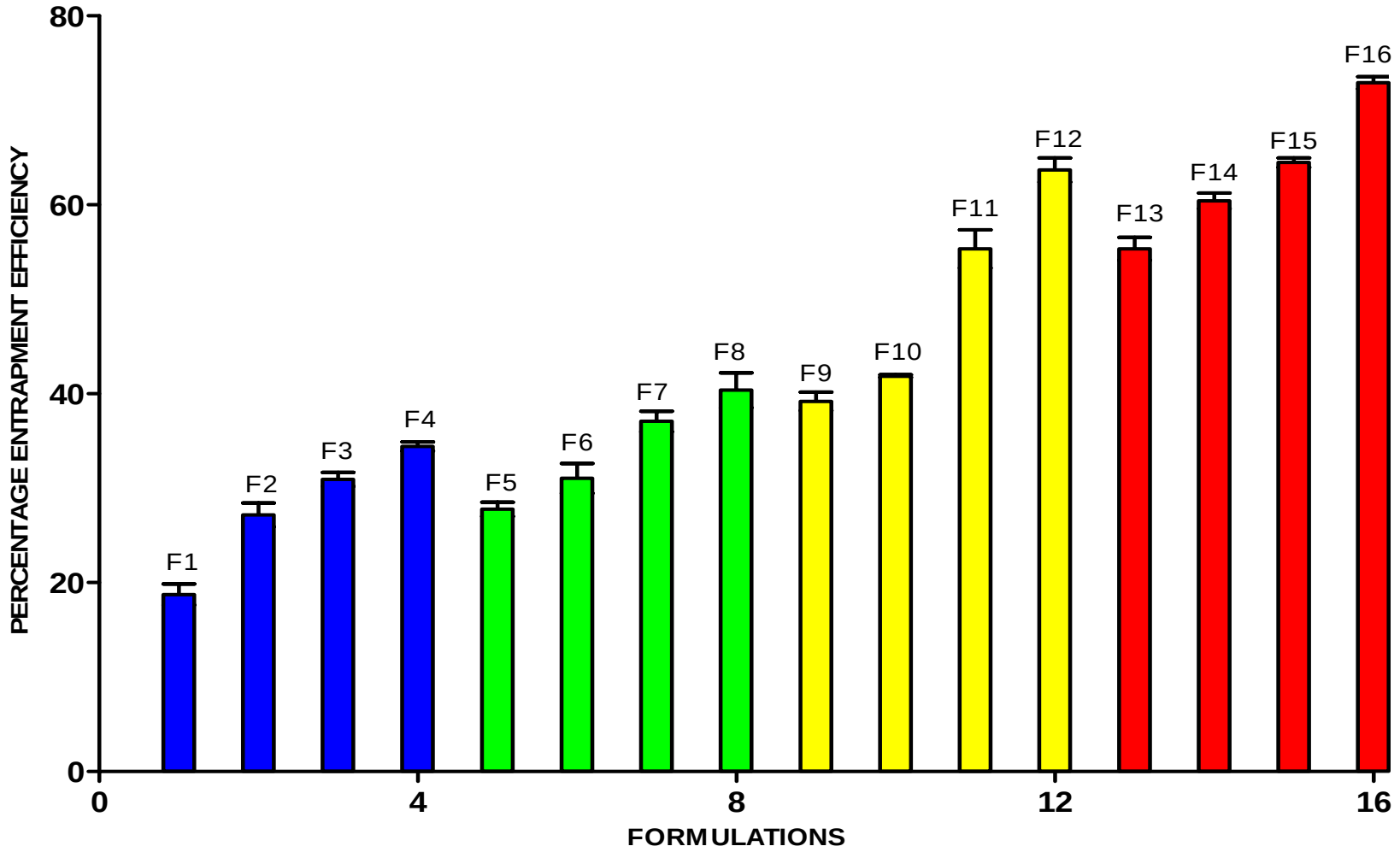


FIGURE- 7

λMAX OF DICLOFENAC POTASSIUM

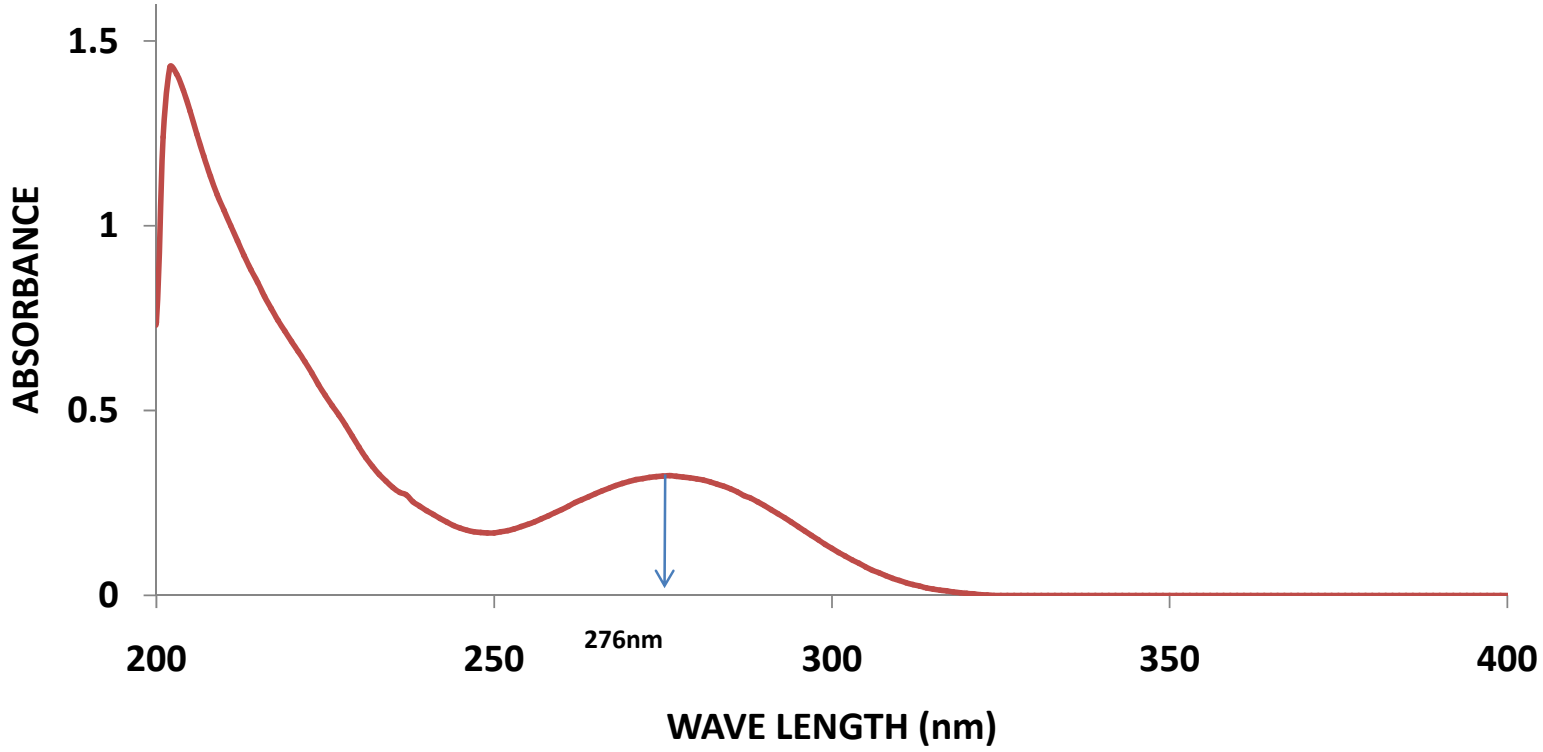


FIGURE- 21

SKIN RETENTION OF DRUG FROM VARIOUS FORMULATIONS

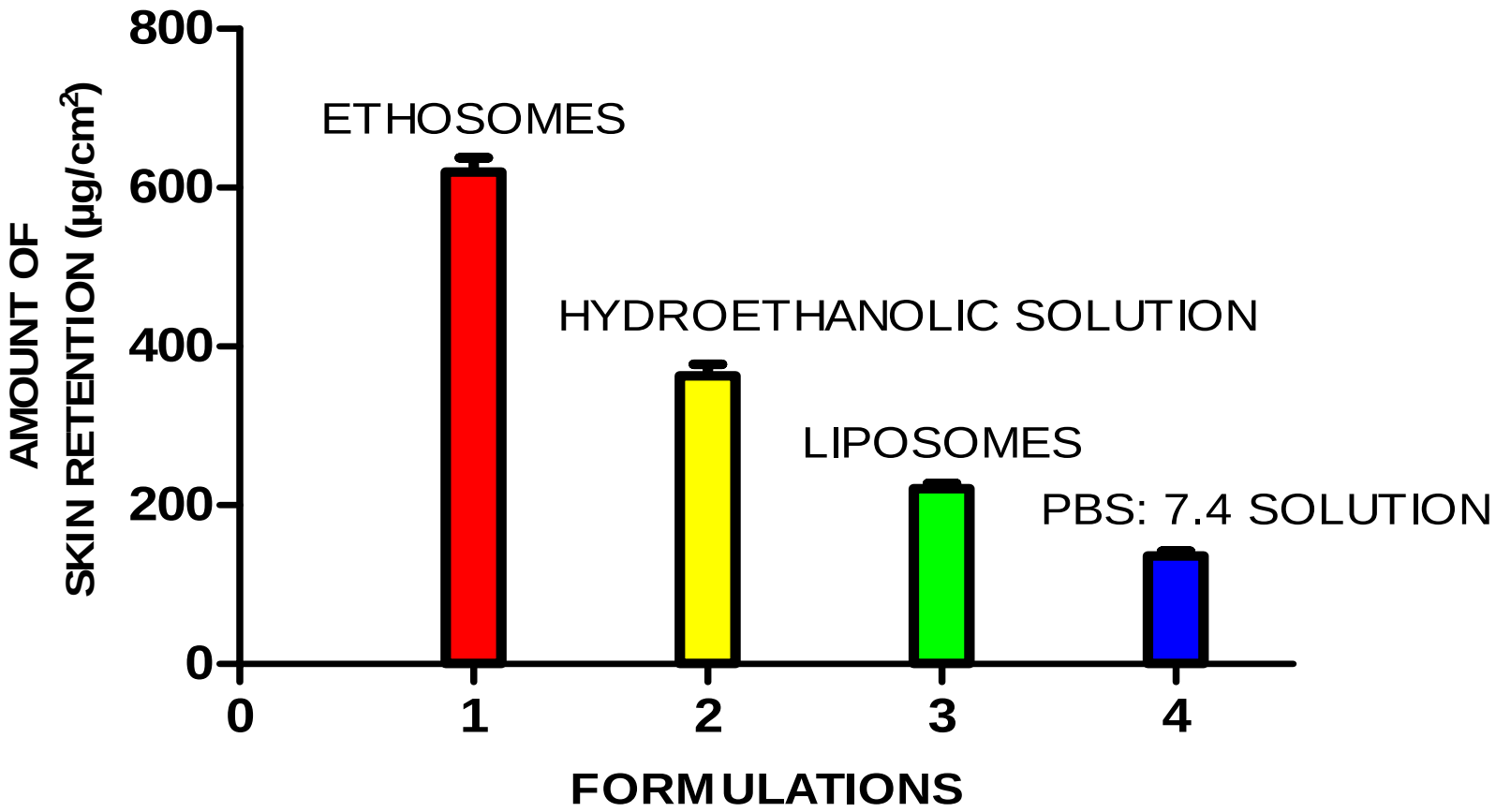


FIGURE- 19

VESICULAR SIZE AND SIZE DISTRIBUTION OF ETHOSOMES AND LIPOSOMES

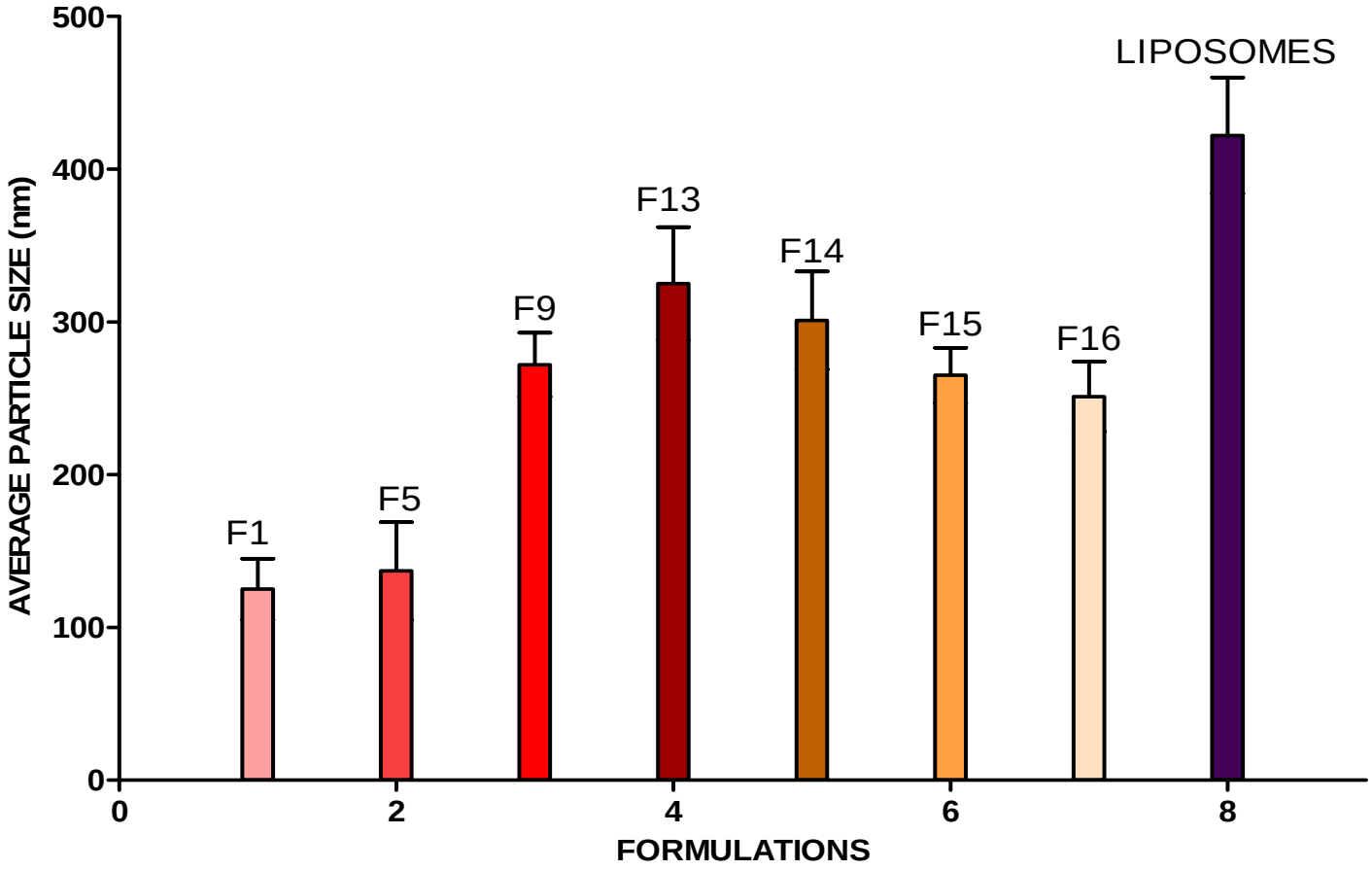
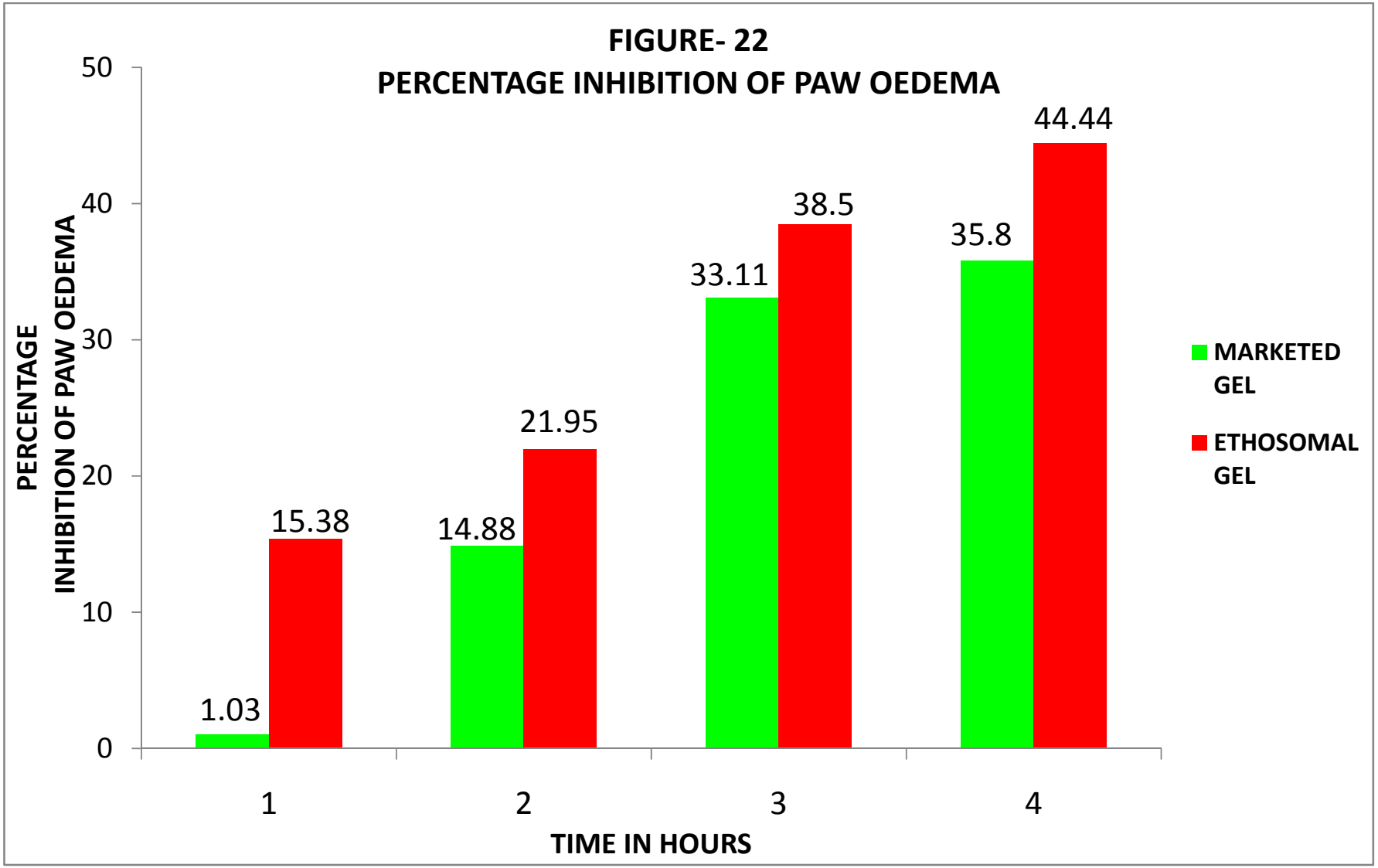


FIGURE- 22

PERCENTAGE INHIBITION OF PAW OEDEMA



CHAPTER-IX

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